VOLUME 59 *April 1949* NUMBER 4

THE AMERICAN REVIEW OF TUBERCULOSIS

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

CONTENTS

Diagnosis and Treatment of Lung Tumor. RALPH ADAMS 353

Observations on the Causes of Dyspnea in Chronic Pulmonary Granulomatosis in Beryllium Workers. ROBERT A. BRUCE, FRANK W. LOVEJOY, JR., GEORGE B. BROTHERS AND TULIO VELASQUEZ 364

The Role of Pulmonary Cavitation in the Development of Bacterial Resistance to Streptomycin. W. L. HOWARD, F. MARESH, E. E. MUELLER, S. A. YANNITELLI AND C. E. WOODRUFF 391

Sensitivity of Tubercle Bacilli to Streptomycin. The Influence of Various Factors upon the Emergence of Resistant Strains. KIRBY S. HOWLETT, JR., JOHN B. O'CONNOR, JOSEPH F. SADUSK, JR., WILLIAM E. SWIFT, JR., AND FREDERICK A. BEARDSLEY 402

Coexisting Disseminated Coccidioidomycosis and Tuberculosis. GEORGE M. FIRESTONE AND ELLIS S. BENSON 415

Viability of Tubercle Bacilli in Embalmed Human Lung Tissue. GORDON M. MEADE AND WILLIAM STEENKEN, JR. 429

Acquired Resistance of *M. Tuberculosis* to Streptomycin *in vitro* and its Behavior in Experimental Infections. E. W. EMMART, W. T. MCCLOSKY, M. I. SMITH AND J. E. LIEBERMAN .. 438

Routine Examination of Sputum for Acid-fast Bacilli. DAVID G. FREIMAN AND MAX PINNER .. 449

A Study of the Bacteriostatic and Bactericidal Activity of Promin, Diasone, Sulfathiazole, Sulfaguanidine and Streptomycin on the H-37 V Strain of *M. Tuberculosis*. K. T. SASANO 461

Obituary: JAMES ALEXANDER MILLER, 1874–1948 467

Abstracts 49

ANNUAL MEETING • DETROIT, MICHIGAN
MEDICAL SESSIONS
MAY 3–5, 1949

Published Monthly at Mount Royal and Guilford Avenues, Baltimore 2, Maryland by the National Tuberculosis Association • Business Off., 1790 Broadway, New York 19, N. Y.

Copyright 1949, by the National Tuberculosis Association

PRINTED IN U. S. A.

THE AMERICAN REVIEW
OF
TUBERCULOSIS

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY

EDITOR-IN-CHIEF
ESMOND R. LONG
Philadelphia, Pa.

MANAGING EDITOR
WALSH McDERMOTT
New York, N. Y.

EDITORIAL BOARD

EMIL BOGEN, Olive View, Calif.
W. EDWARD CHAMBERLAIN, Philadelphia, Pa.
HALBERT L. DUNN, Washington, D. C.
KIRBY S. HOWLETT, JR., Sheldon, Conn.
HERBERT C. MAIER, New York, N. Y.
WILLIAM P. SHEPARD, San Francisco, Calif.
SIDNEY J. SHIPMAN, San Francisco, Calif.
JOHN D. STEELE, Milwaukee, Wisc.
C. EUGENE WOODRUFF, Northville, Mich.

NOTICE TO SUBSCRIBERS AND CONTRIBUTORS

THE AMERICAN REVIEW OF TUBERCULOSIS is published by the National Tuberculosis Association and issued monthly about the 1st of the month. THE AMERICAN REVIEW OF TUBERCULOSIS is the Official Journal of the American Trudeau Society. A volume includes six numbers and begins with the January and July numbers.

Subscriptions: The subscription price of the REVIEW is $8.00 for the calendar year. Checks should be made payable to Collier Platt, Treasurer.

Character of the Review: The REVIEW consists of two main parts, namely, (1) original articles and (2) abstracts. The original part is published monthly, its size depending upon the amount of manuscript in hand. Abstracts will appear as the amount of material warrants. Each part will be paged separately, to permit permanent separate binding upon the completion of a volume.

Manuscripts: "The REVIEW invites the submission of manuscripts on any phase of tuberculosis, non-tuberculous pulmonary diseases, and related subjects of interest. Illustrations for reproduction must be of first class quality. It is essential that photographs and reproductions of roentgenograms clearly furnish all the detail the author wishes illustrated. Articles are accepted for publication with the understanding that they are contributed solely to THE AMERICAN REVIEW OF TUBERCULOSIS."

Manuscripts should be sent to the office of the Managing Editor, Dr. Walsh McDermott, 1790 Broadway, New York 19, N. Y. They should be in English, typewritten on one side of the page only and with wide spacing and margins. In order to hasten the process of reviewing and editing, it is requested that each original manuscript be accompanied by a duplicate copy. This will be returned to the author when a final decision regarding publication is made. The manuscripts should be mailed flat and transmitted by first-class mail with postage for return if not available. Authors should exercise particular care in the preparation, notation and description of figures, charts and tables.

The publishers will not be responsible for manuscripts, illustrations, etc., lost in transit. In order to save expense for authors' corrections in proof, manuscript should be carefully revised by the author before submission.

Abstracts: Authors wishing to have abstracts of their papers appear in ABSTRACTS OF TUBERCULOSIS will facilitate their publication by sending concise abstracts or reprints to the Managing Editor.

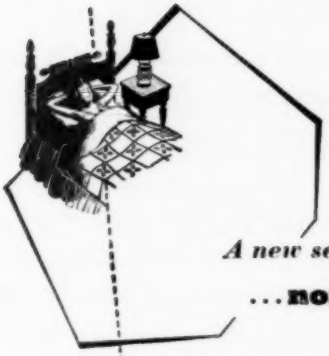
Reprints: Fifty reprints without covers of articles will be furnished to authors free of charge when requested in advance. A table showing cost of additional reprints, with an order blank is submitted with proofs to the authors.

Single Copies: The price of single copies of this number of THE AMERICAN REVIEW OF TUBERCULOSIS is one dollar postpaid.

Advertising: Rates will be furnished by THE AMERICAN REVIEW OF TUBERCULOSIS, 1790 Broadway, New York 19, N. Y., on request.

"The Publishers reserve the right to decline any advertising submitted and to censor all copy. Acceptance of an advertisement does not constitute official endorsement of the product advertised."

Entered as second-class matter March 9, 1917, at the post-office at Baltimore, Md., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in section 1103, act of October 3, 1917. Authorized on July 2, 1918.



A new sedative-hypnotic

*...**not** a barbiturate*

Presidon, a new quick-acting, mild sedative-hypnotic for insomnia and nervous tension, is a pyridine derivative chemically different from the barbiturates, bromides and ureides. Therapeutically it differs in the low incidence of usual by-effects. Clinical trials show that needed relaxation or sleep is obtained without likelihood of drowsiness on awakening, "hangover," excitation or headache. Available in scored 0.2 Gm tablets, bottles of 20 and 100.

HOFFMANN-LA ROCHE INC. • NUTLEY 10 • N. J.

Presidon®

'Roche'

The Influence of Timing On Protein Utilization

The biologic importance of complete protein in the daily diet takes on new significance in the light of recent research on the influence of timing upon protein utilization.[1,2]

Protein-depleted animals, maintained on an otherwise adequate diet, rapidly regained weight when fed their complete ration of the ten essential amino acids. When, however, these ten amino acids were not provided simultaneously—five were given at one feeding and the other five in a feeding two hours later—recovery did not occur.

These studies strongly suggest that efficient protein utilization requires simultaneous absorption of the indispensable amino acids. New emphasis is thus given to the rationale of routinely including biologically complete protein in all meals to assure optimal utilization of all the protein ingested.

Meat is an outstanding source of complete protein. Regardless of cut or kind, whether fresh, cured, or canned, all meat contains the ten indispensable amino acids capable of satisfying every protein need of the organism. And, all meat is of excellent digestibility—from 96 to 98 per cent.

---

[1]Cannon, P. R.; Steffee, C. H.; Frazier, L. J.; Rowley, D. A., and Stepto, R.C.: The Influence of Time of Ingestion of Essential Amino Acids upon Utilization in Tissue Synthesis, Fed. Proc. 6:390, 1947.

[2]Geiger, E.: The Role of the Time Factor in Feeding Supplementary Proteins, J. Nutrition 36:813 (Dec. 10) 1948.

The Seal of Acceptance denotes that the nutritional statements made in this advertisement are acceptable to the Council on Foods and Nutrition of the American Medical Association.

American Meat Institute

Main Office, Chicago...Members Throughout the United States

A Significant Advance in ANTIBIOTIC THERAPY

Note these five favorable attributes of Dihydrostreptomycin Merck

(1) Low incidence of vestibular disturbances

(2) Significantly less toxic

(3) Less frequent allergic manifestations

(4) Highly purified

(5) Undiminished antibacterial activity against *Mycobacterium tuberculosis*

A NEW, highly purified antibiotic, chemically distinct from streptomycin, with greatly reduced neurotoxicity, Dihydrostreptomycin Merck is especially useful in cases requiring relatively high dosage, such as miliary tuberculosis and tuberculous meningitis.

It can be used interchangeably for intramuscular therapy with Streptomycin Calcium Chloride Complex Merck or other forms of streptomycin.

Descriptive literature is yours for the asking.

LOW INCIDENCE OF EIGHTH CRANIAL NERVE DAMAGE

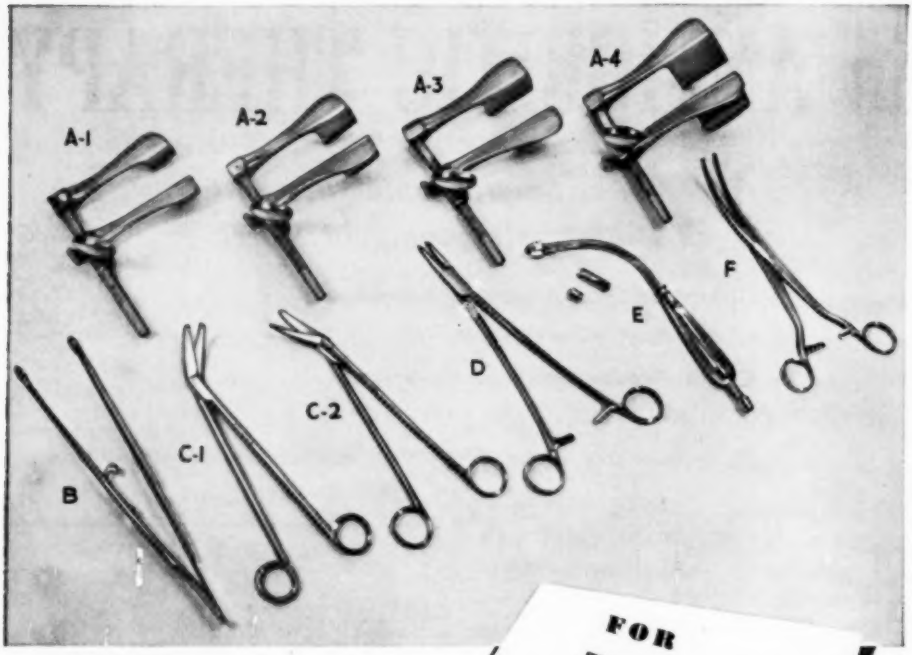
DIHYDROSTREPTOMYCIN MERCK

(supplied as the sulfate)

MERCK

MERCK & CO., Inc. *Manufacturing Chemists* **RAHWAY, N. J.**

INSTRUMENTS OF AUTHENTIC DESIGN



FOR THORACIC SURGERY

The following thoracic instruments are the special design of William Francis Rienhoff, M.D., Baltimore, Md.

A1 P16962 Rienhoff Rib Retractor, for children and small adults.

A2 P16963 Same, for women and men of slighter build.

A3 P16964 Same, for large and corpulent patients.

A4 P16965 Same, for heavy, obese patients.

B P16977 Rienhoff Ring-End Tissue Forceps, with ratchet. Rienhoff Ring-End Tissue Forceps. Same as P16977, but without ratchet. (Not illustrated.)

C1 P16979 Rienhoff Dissection Scissors, angular blades, curved to right.

C2 P16980 Same, blades curved to left.

D P16982 Rienhoff Needle Holder, gold-plated for quick identification.

E P16983 Rienhoff Suction Tube, with 3 removable tips for pointed, dissection or round-end suction.

F P16985 Rienhoff Hemostatic Lung Clamp (1x2 teeth).

Order Pilling instruments direct,

or write for further information to:

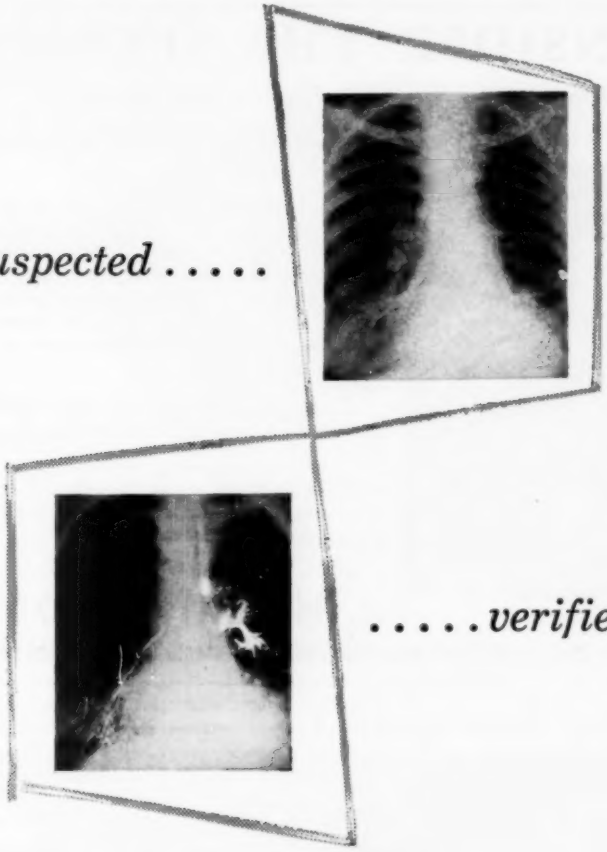
GEORGE P. PILLING & SON CO.
3451 Walnut Street
Philadelphia 4

A Standing Invitation:
When in Philadelphia, visit our new salesrooms. Free parking on our private lot.

INSTRUMENTS BY Pilling

PILLING FOR PERFECTION in surgical instruments

suspected . . . . .



. . . . . verified

. . . Lipiodol*

completes the diagnostic picture

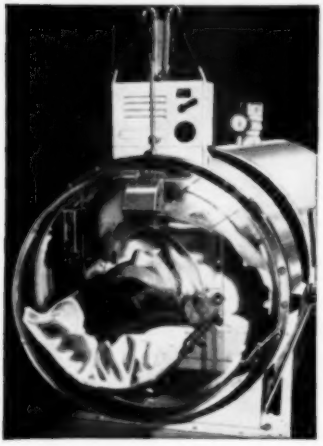
From its introduction to the present, Lipiodol has remained a contrast medium of choice . . . notable for these properties: **1** 40% iodine content firmly bound in poppyseed oil, insures uniform radiopacity **2** viscosity characteristics produce clear delineation without excessive "pooling" **3** exclusive formulation does not involve use of chlorine or its derivatives **4** its blandness insures minimal irritation to mucous membranes.

100th Anniversary
E. Fougera
1849-1949
A Century of Service to Medicine

*Lipiodol (Iodized oil, U. S. P.) is the registered trade-mark for the original product created by Lafay. This product alone can bear the name Lipiodol. Made in the U. S. A. E. Fougera & Co., Inc., New York, N. Y. Canadian Distributors: Vinant, Ltd., Montreal, Canada.

CONSIDER THE ECONOMY

In Addition to the Service to Human Individuals



if you can obtain return to normal life of patients who would otherwise be indefinitely hospitalized . . . operated on . . . nursed . . .

Not all cases can be so fortunate, but more than half of those treated in Emerson Lung Immobilizers by Barach's method of total lung rest have shown sucessful results—"disappearance of cavity with negative sputum", "arrest of their disease with ability to work", etc. And these were cases of advanced, bilateral, cavitary tuberculosis which had previously proved resistant to usual measures.

Immobilization therapy is not expensive, is generally not long, and does not preclude other procedures.

It is worth noting that even small county sanatoria, starting with single immobilizers, have ordered second and then third machines, because of this economy in dollars—and lives.

For information and reprints, write:

J. H. EMERSON CO.

22 COTTAGE PARK AVENUE, CAMBRIDGE 40, MASSACHUSETTS

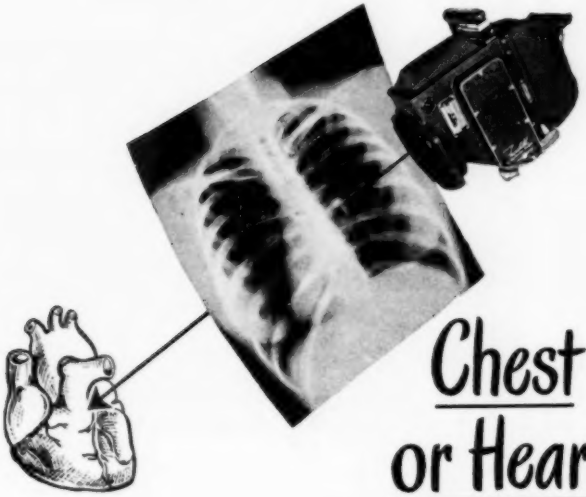
EUDOWOOD SANATORIUM

Towson, Maryland

A modern, thoroughly equipped institution for the treatment of tuberculosis. Located on a large 350-acre farm in the beautiful, healthful Maryland countryside. All city conveniences . . . eight miles north of Baltimore . . . one mile east of Towson, Maryland.

Private rooms with adjoining baths . . . $7.00 and $8.00 per day, including general nursing and medical attention. *For further information, address:*

DR. WILLIAM A. BRIDGES, *Superintendent*
Eudowood, Towson 4, Maryland



Chest
or Heart

WITH ONE 70mm FLUORO-RECORD CAMERA

VERSATILE OPERATION: Fairchild's 70mm Fluoro-Record Camera—*which is now available on leading X-ray equipment*—can be used for mass X-rays . . . routine chest work . . . occasional test or experimental shots . . . and angiocardiography *without* expensive accessories or unnecessary duplication of equipment.

SPEED: 1200 or more X-ray exposures can be taken in a single day. Routine or test shots can be processed immediately after exposure. Angiographic shots can be taken at the rate of 16 or more every 10 seconds depending on the size of the patient to provide an adequate record for accurate diagnosis.

ECONOMY: A 100 ft. roll of film produces up to 450 negatives. The cost is approximately 3½¢ per frame. Double 2½ x 3 inch or 2½ x 2½ inch cut film strips cost less than 3¢ per frame.

CONVENIENCE: 70mm film—which has proven to be an ideal size for routine and mass radiography—is sufficiently large to permit easy reading without high magnification.

The same precisionized electronic and mechanical skill—that ranks Fairchild Aerial Cameras and Navigational Instruments with the world's finest—also produces 70mm FLUORO-RECORD Cut Film Cameras . . . Cut, Roll and Stereo Film Viewers . . . Roll Film Developing and Drying Units. Also the Chamberlain X-ray Film Identifier. Available thru your X-ray Equipment Supplier.

Fairchild
CAMERA AND INSTRUMENT CORPORATION

88-06 VAN WYCK BOULEVARD, JAMAICA 1, NEW YORK

OAK RIDGE SANATORIUM

GREEN SPRINGS, OHIO

for
TUBERCULOSIS

•

Diagnosis
Treatment

•

SITUATED in the beautiful springs country of Northern Ohio, this modern Sanatorium offers not only up-to-date treatment for all forms of Tuberculosis but a setting of utmost beauty and restfulness for the convalescent. General Hospital Facilities with complete Surgery . . . Modern Steam-heated Rooms . . . Graduate Nurses . . . Personal Care for Every Patient.

Natural Mineral Spring
(8,000,000 gallons per day.)
Artesian Well

Reasonable rates

PAUL M. HOLMES, M.D., Medical Director
JOHN J. GEDERT, M.D., Resident Physician
GEO. S. BOWERS, M.D., Internist
ELEANOR BLIVEN, R.N., Supt. Nurses
ALEX C. JOHNSON, Pres. & Gen. Mgr.
M. M. RIDDLE, M.D., Eye, Ear, Nose and Throat
WM. NEILL, M.D., Thoracic Surgeon
OTTO MUHME, M.D., Thoracic Surgeon

Bindings for Review Copies

THE AMERICAN REVIEW OF TUBERCULOSIS will bind current and back numbers of the REVIEW in standard uniform cloth binding.

Cost of binding, $3.00 per binding
Transportation and wrapping charges additional.

Please fill in the order below, detach this page and mail to:

AMERICAN REVIEW OF TUBERCULOSIS
1790 Broadway, New York 19, N. Y.

Please fill my order for binding my REVIEW volumes in the manner checked, supplying all the missing numbers.

☐ Original Articles and Abstracts of each volume separately at $6.00 per volume for binding.
☐ Articles and Abstracts for one volume in one binding at $3.00 per volume for binding.
☐ Articles and Abstracts for entire year bound separately at $3.00 per binding.

I desire volumes*.....bound and have sent to you at the above address under the date of.....by.....(Pp. or Express).....Volumes.....

NAME.....

ADDRESS.....

* If you order Volumes XI and XII bound and wish them combined, check here ☐

The dihydro form of streptomycin

Dihydrostreptomycin Squibb
HYDROCHLORIDE

PERMITS HIGHER DOSAGE FOR MORE PROLONGED PERIODS

WHAT IS IT?

A potent antibiotic compound derived from streptomycin by reduction with hydrogen.

WHEN IS IT INDICATED?

Like streptomycin, as an adjunct to other measures in tuberculosis.

HOW DOES IT ACT?

The antibacterial activity of Dihydrostreptomycin usually parallels that of streptomycin in tuberculosis. Resistant strains of organisms appear to develop as rapidly as with streptomycin.

WHAT ARE ITS ADVANTAGES?

Dihydrostreptomycin is significantly less neurotoxic than streptomycin and hence can be given in larger doses and for more prolonged periods. In addition, patients showing allergic reactions to streptomycin have been able to continue with the dihydro form.

HOW IS IT ADMINISTERED?

Only intramuscularly, pending further clinical studies.

WHAT IS THE DOSAGE?

Daily doses of 2 grams of Dihydrostreptomycin Squibb may be given safely for periods equal to those in which streptomycin has been restricted to 1 gram a day, provided there is no renal dysfunction. Average dosage—1 to 2 grams daily in divided doses every 12 hours.

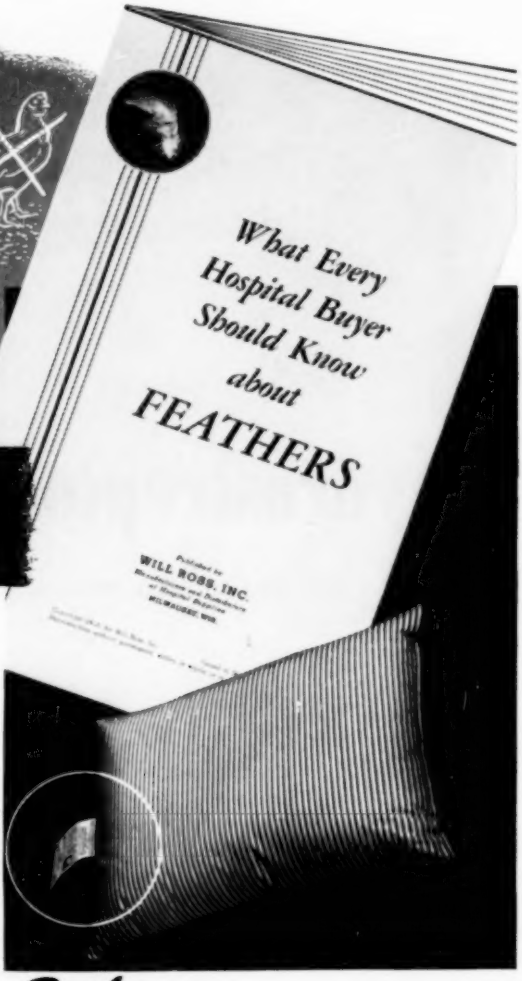
HOW SUPPLIED?

20 cc. vials containing the equivalent of 1 Gm. streptomycin base
50 cc. vials containing the equivalent of 5 Gm. streptomycin base

E. R. Squibb & Sons, New York 22, N. Y.

SQUIBB *a leader in antibiotic research and manufacture*

Revised Edition



"What Every Hospital Buyer Should Know About *Feathers!*"

THIS important booklet, originally published in September 1939, has been revised and brought up to date. Over the years it has not only rendered a real service to hospitals but because it is full of factual information, liberally illustrated with photographs and diagrams, it has been requested by hundreds of Universities, Libraries, Federal and State Government Departments and others outside of the hospital field.

It discusses types of feathers, where feathers come from, how they are processed, how to identify and judge feathers, how to analyze feather mixtures, the best construction of hospital pillows, how to care for pillows, State and National bedding laws, the meaning and importance of the "New York Label" and other important facts about feathers and pillows.

A copy of this Revised Edition has recently been mailed to most hospitals in the United States. If, for any reason, your copy did not reach you, or if you want an additional copy, drop us a line. We will gladly mail one.

3 things EVERY HOSPITAL BUYER SHOULD KNOW ABOUT WHITE KNIGHT PILLOWS!

1 They are manufactured to our own very rigid specifications (developed as a result of years of study of feathers and feather pillows) to meet all hospital requirements.

2 They are "Sanitized" — a chemical treatment that retards germ and mold growth, resists odors caused by heat and perspiration, protects from breakdowns through the action of bacteria and resists moth damage.

3 They all carry the New York Label — an effective guarantee against misbranding.

WILL ROSS, Inc.

Manufacturers and Distributors of Hospital and Sanatorium Supplies and Equipment

MILWAUKEE 10, WISCONSIN

DIAGNOSIS AND TREATMENT OF LUNG TUMOR[1, 2]

RALPH ADAMS

(Received for publication November 19, 1948)

INTRODUCTION

Carcinoma of the lung accounts for between 6 and 8 per cent of all cancer and approximates the frequency of gastric carcinoma. It is, therefore, a common disease which every general physician may expect to encounter in his practice. Usually the diagnosis is easily made by observation and analysis of the symptomatology which differs little from that of numerous other thoracic diseases.

The results of treatment are comparable to those of other visceral cancers and much progress has been made in treatment in the past fifteen years, both in respect to earlier diagnosis and improved technique. There has been a marked reduction in the immediate operative mortality, which formerly was very high but now is less than 5 per cent. The chief problem, in the present state of knowledge or lack of knowledge of cancer causation is that common to all malignant disease, i.e., earlier diagnosis. The available surgical procedures are now about as radical as is feasible or practically applicable. Some improvement still is possible in short term results on poor risk patients by further refinement in methods of preparation and of after care.

The content of this paper is limited to a statement of working policies in the detection of cases of lung tumor and the care of patients with such disease; in other words, practical diagnosis and treatment based on my own observations and experience. It excludes detailed statistical reporting of factual data as garnered from records of some 385 microscopically proved cases. It also excludes minutiae of technical detail as both these items are of especial concern only to those primarily interested in this single disease.

MEASURES TO MINIMIZE INCIDENCE

This topic is mentioned only because there is nothing definite to report, nor is there likely to be until the cause of cancer is discovered. Cigarette smoking has been incriminated, but not proved. Neither has chronic inflammation or irritation been shown to be causative, as is suspected in relation to carcinoma of the cervix. The one exception to complete ignorance of cause is the established fact of increased incidence in miners exposed to radioactive ores. The reason for the 4 to 1 male predisposition also is not explained.

DIAGNOSIS

Value of Population Survey

Large scale radiographic screening has revealed some type of thoracic lesion in approximately 1 per cent of the segments of population examined, but only

[1] From the Good Samaritan Hospital, Woodbury, Tennessee.

[2] Presented at the Southern Tuberculosis Conference, Savannah, Georgia, October 1, 1948.

a fraction of these cases have been shown or even suspected of having carcinoma. The group in which carcinoma is most prevalent is not often reached by the screening policies adopted for such surveys, which are designed to find tuberculosis and are directed toward the younger age groups. However, an occasional case of carcinoma is found.

Furthermore, periodic health examination will not find the silent malignant lesion in the lung, unless: (*1*) it is accompanied by roentgenologic examination and the growth is in the lung parenchyma or encroaching on the bronchial lumen; and (*2*) such examination is repeated more often than is necessary in Public Health control programs. The three most valuable approaches to discovery of really early carcinoma of the lung are: (*1*) education of the public to see a physician for any cough of over two weeks' duration; (*2*) education of the profession to inquire for symptoms and at least fluoroscope every new patient and make chest films on fluoroscopic indication or presence of symptoms; (*3*) stop the practice of symptomatic treatment of cough or pleurisy without evaluation of cause.

Symptomatology

In contrast to carcinoma of the stomach, lung cancer usually causes early symptoms, but they are often overlooked. They are so ordinary in character that both the patient and the physician are likely to disregard them in early stages. As determined from study of one group of 157 consecutive, microscopically proved cases (1), the early symptoms in the order of frequency are:

Cough: Cough is present in 93 per cent of the patients with lung cancer. In the early states it is dry or irritative, but soon becomes productive of mucoid or purulent sputum. Most often it is attributed to cigarette smoking or a mild cold.

Pain: Almost every patient has a different concept of pain, depending upon his temperament and racial and social background. Early carcinoma of the lung rarely causes true pain, but some degree of pleuritic or vague thoracic discomfort will be admitted in slightly over half the patients.

Sputum: The character of sputum in carcinoma of the lung is related closely to secondary infection. Very early in the disease true mucoid sputum, apparently caused by mucosal irritation or stimulation by the growth, is encountered. Later the sputum usually becomes purulent, but not foul or fetid, unless there be bronchial obstruction or tumor necrosis.

Hemoptysis: Blood spitting which occurs each day, every day, persistently over a period of time, is caused almost solely by carcinoma. However, hemoptysis in carcinoma also may be sporadic or intermittent.

Wheeze: This symptom or sign is not to be confused with asthma. It is an indication of bronchial encroachment to a point of incomplete occlusion and its intermittent character in early stages is explained by the presence or absence of secretion in the narrowed bronchial lumen. In combination with one or more of the preceding symptoms, it provides strong presumptive evidence of bronchial occlusion caused by tumor unless there be a history of foreign body inhalation.

Confirmation of localized obstruction may be furnished by roentgenologic observation of mediastinal shift or localized compensatory emphysema.

A late symptom of *dyspnea* is closely correlated to *pleural effusion*. Bloody effusion is the type commonly found, but is not the infallible indication of malignancy once thought, because it can also be caused by infarct or an inflammatory reaction. Furthermore, effusion also occurs in nonsanguinous form from pleural involvement by cancer.

Severe pain, as distinguished from mild pleural discomfort, should be regarded as a consequence of brachial plexus, chest wall, or mediastinal invasion. Relief from such pain is infrequently obtained by direct operative procedures, including removal of ribs, intercostal neurectomy, or lobar excision. Occasionally, X-ray therapy is of transient benefit as are the nitrogen mustards. On the whole, if the pain really remains intractable and beyond the range of control by medication, one must resort to chordotomy or frontal lobotomy. The latter procedure has given very gratifying results to a few of our cases but, because of the attendant personality changes, must be embarked upon only after full consultation with the neurosurgeon, the personal physician, and the family.

Hoarseness: The appearance of hoarseness or huskiness of voice suggests paralysis of the recurrent laryngeal nerve from mediastinal extension of the growth. Occasionally the voice will return almost to normal after a few weeks of hoarseness, giving the patient and perhaps the physician a false sense of security, even though the vocal cord remains paralyzed. This phenomenon, when it occurs, is related to laryngeal compensation. In most instances the voice remains permanently husky. One must beware of deciding forthwith that a patient has inoperable cancer of the lung when he has been shown to have an abnormal shadow in the chest and a paralyzed vocal cord. We have now encountered 3 cases with this situation in which cancer was not present and the patients made a complete recovery following appropriate surgery. One was a dermoid cyst with infection; one was a teratoma with surrounding inflammatory reaction; and one was a substernal goiter into which there had been recent hemorrhage.

Weight loss and weakness occur in later stages of the disease and direct attention to a careful search for peripheral nodes and enlarged liver or other signs of distant metastasis.

Physical Signs

The physical signs of lung cancer are related solely to bronchial obstruction and sequelae or to extension of the growth. The presence of a tumor mass itself is rarely detectable by physical examination. One may find the signs of pulmonary infection or atelectasis or pleural effusion. Paralysis of the phrenic nerve or the recurrent laryngeal nerve are detectable by physical observation, as previously mentioned. Every case should have careful, methodical palpation of the supraclavicular fossae and axillae for nodes. In instances where pain is a complaint, it is important to palpate meticulously the intercostal spaces and muscles about the scapula for signs of tenderness or thickening from tumor invasion.

Definitive Diagnostic Tests

The observations thus far mentioned are limited to recognition that disease is present, but are not definitive, for they may be encountered in various types of disease of the lung. Further study should include as many of the following additional procedures as are required for diagnosis.

1. Examination of the vocal cords with a laryngeal mirror. This is not a specialists' procedure and the simple technique can be mastered by anyone with a little practice.

2. Fluoroscopy. This should be a part of every general examination, and from it one gains specific information concerning diaphragmatic movement and type of movement. On fluoroscopy it is possible to detect immediately paradoxical movement or high stationary position of the diaphragm, secondary to a paralyzed phrenic nerve from tumor extension into the anterior mediastinum. The phrenic nerve, however, can be paralyzed by inflammatory reaction or by the pressure of benign lesions such as an aneurysm of the aortic arch. One observes the position of the mediastinum; whether there is shifting with breathing; whether the shift is toward or away from the lesion; and whether the position of the mediastinum is variable or fixed. Localized emphysema is sought and the respiratory phase in which it appears is noted. Partial bronchial obstruction causes localized expiratory wheeze and emphysema in combination. From fluoroscopy one may better determine what special radiographic views will best record the lesion. Finally, one learns whether an abnormal mass is of fixed size, pulsatile, or expansile.

Radiographic films are obligatory in the presence of symptoms or signs even if fluoroscopic observations are normal because the problem at this stage is one of differential diagnosis. Fluoroscopy alone may fail to reveal the early infiltrative, or exudative, or very small lesion, because of limited optical resolving power. It is beyond my competence to speak authoritatively on the purely technical phases of radiology, but the following common errors in making radiographs are mentioned because they are responsible for some errors in diagnosis: (*1*) omission of the lateral films which often assist in detection of pathology behind the heart or near the mediastinum and permit positional localization to a pulmonary segment, division or lobe; (*2*) failure to obtain oblique views of lesions requiring differentiation from anomalies or morbid processes in the heart or great vessels; (*3*) simple, technical inadequacy, such as overexposure and underdevelopment, gross underexposure or development in solutions that are too hot.

Supplementary films with heavy exposure or with the Buckey diaphragm should be made for lesions of marked or extensive density in order to detect an internal cavity or to visualize the bronchial lumen, and to study the ribs, pleura and mediastinum for irregularity. Laminograms occasionally are helpful but are more useful in the study of tuberculosis.

Radiographic Evidence

The most direct primary evidence consists of the demonstration of a tumor mass. If circular in form, it is likely to lie in the periphery, even though it may

appear to be situated close to the hilus on a postero-anterior film. A lateral film will complete definition of the location of the mass. If multiple shadows of round masses are seen, metastatic disease to the lungs is naturally suspected. If the mass lies in the periphery, is round, and is more than 4 cm. in diameter, either adenocarcinoma or solitary metastasis from a hypernephroma is to be suspected, and intravenous pyelograms should be done. The other primary evidence consists of the presence of a blocked bronchus with an ovoid shadow of tumor projecting into the bronchial air column above the obstruction. This is sometimes seen on a plain film, more often on a Buckey film, and most often on a laminograph.

The secondary evidence of the presence of a tumor usually consists of radiographic and the other signs of pulmonary atelectasis or infection which are related to variable degrees of bronchial encroachment and obstruction. Important information, which suggests the occurrence of subcarinal lymph node extension, is afforded by a broadened or distorted tracheal bifurcation.

Certain axioms are of aid in radiographic interpretation, remembering that they are statements of clinical experience and not of mathematical law. Localized emphysema and wheeze in an adult are usually caused by a tumor. A widened carina indicates disease in the mediastinal nodes and hence is extremely likely to indicate the presence of tumor. A lung abscess with thick wall (greater than 1 cm.) and nonfetid sputum is presumptive evidence of carcinoma. A fluid "level" with upward convexity is caused by central necrosis of a tumor mass having bronchial communication. Radiographic reports reading "suggest recheck in two months to rule out carcinoma" are never justified on the basis of haphazard or incomplete study, and may cause needless delay in diagnosis.

Summary of radiographic study: Radiographic examination excels all other methods in the detection and recognition of lung cancer if done completely and properly by persons of experience and competence.

Examination of the Sputum for Cancer Cells

Examination of the sputum for cancer cells is an old method which has been in use for many years. It has recently been repopularized and increased in value by the work and writing of Papanicolaou. Such examinations should be performed in all cases under suspicion of carcinoma of the lung, just as a search for tubercle bacilli in the sputum is made in all cases under suspicion of pulmonary tuberculosis. Experienced observers make very few false positive reports, but the report must be correlated with other observations. Negative reports or findings have little significance. The test is of considerable value when positive, but is not as yet as definitive as the evidence provided by examination of material obtained by a direct biopsy.

Thoracentesis is indicated in cases in which a pleural effusion is detected on clinical or radiographic examination. Aspiration is best done with a short beveled needle of number 18 gauge lumen, 2 inches in length. One might easily recite a series of cases seen with complications of pleural or pulmonary infection, hemorrhage or spread of disease, which were direct sequels to the injudicious use of a large, long, bayonet-pointed needle.

The fluid obtained from undiagnosed cases should be studied by smear; by appropriate cultures for aerobic and anaerobic microorganisms, including the fungi; determination of specific gravity and cell count; concentration and examination for tubercle bacilli and cancer cells. In cases of known carcinoma, when the question is reduced to one of malignant pleuritis, study of the concentrate by cytologic methods alone is sufficient. Although usually true, the long held tenet that the presence of pleural effusion with carcinoma invariably indicates inoperability has had to be altered. Similar effusions may be caused by associated or secondary parenchymal infections. A three year respite from symptoms has been observed after resection of a pulmonary carcinoma in which there was a massive pleural effusion.

On rare occasion the adjuvant use of a thoracoscope is helpful. Likewise, replacement of fluid with a mantle of air now and again will assist in demonstration of pleural nodularity on radiographic examination.

While considering the question of the passage of instruments through the chest wall, punch biopsy deserves mention. This procedure was formerly more frequently used by us than at present. It may be accompanied by many hazards such as hemorrhage, infection, or spread of carcinoma along the needle tract. Moreover, the findings are frequently negative when carcinoma is known to be present. Punch biopsy should be used only in cases deemed inoperable but on which a microscopic diagnosis is necessary as a guide in nonsurgical treatment.

Bronchoscopy

Next to roentgenologic study, bronchoscopy is the most important definitive procedure in evaluation of the patient with possible lung cancer. Evaluation is used as a broader term than diagnosis. If the bronchoscope is employed solely as a lighted tube through which someone may pass a forceps and excise a piece of abnormal tissue if fortuitously encountered, the value of the endoscopic study is limited in the same fashion as if the roentgenologic study were considered to be completed by the taking of a single postero-anterior chest film.

Many reasons exist for omitting bronchoscopy, including a peripheral location of the lesion, the absence of bronchial obstruction, or the ability to establish a diagnosis and decide upon therapy by other means. The indications for performance of bronchoscopy are symptoms, signs or roentgenologic findings of bronchial encroachment or obstruction.

The endoscopist must observe the mobility of the vocal cords and any variation from the normal in the size, contour, shape, flexibility, direction and position of the trachea, carina, primary lobar and segmental bronchi. He must note the appearance of the bronchial mucosa and the location and amount and type of secretion present. Furthermore, he must observe and record the exact position and gross appearance of any tumor found, with particular thought to the bearing of such information on operability. The endoscopist takes a biopsy of suspicious tissue. He examines the upper lobe bronchi, on indication, with optical systems that permit retrograde or right angle visualization. In addition, he may irrigate and aspirate secretions by use of appropriately curved flexible instruments from any segmental bronchus in the lung. Such fresh secretions immediately

prepared in the laboratory may yield well preserved cells from which the pathologist can accurately determine a diagnosis. Negative evidence from endoscopic examination so conducted is of equal importance.

So handled by a seasoned endoscopist, acting as a consultant with clinical acquaintance with the case, one may anticipate on the basis of our experience the following results in approximate figures. Eighty per cent of eventually proved cases of lung cancer will be bronchoscoped. Ninety per cent of the cases bronchoscoped will have the diagnosis established thereby or stated as a positive suspicion from the collateral observations which the bronchoscopist makes. If bronchoscopy is handled as a purely laboratory procedure, to be done by a technician with no knowledge of the case, under remote "pushbutton-like" control from a clinician, the results, I should think, would be less rewarding.

Bronchography rarely should be done in the diagnostic study of lung tumor. The oil may be trapped behind a tumor with production or accentuation of pneumonitis. It carries a slight risk of causing spread of tuberculosis. Moreover, bronchography confuses the radiographic appearance of early inflammatory disease and may make further accurate observation of progress either tedious or inaccurate. Bronchography is a technique for outlining the pattern of disease in bronchiectasis, and even there is only incidentally of diagnostic value.

Thoracotomy

An occasional case is enountered in which no diagnostic approach other than thoracotomy will be decisive. We have formerly made the statement that one rarely learns more about the diagnosis on opening the thorax than was known beforehand, although of course the extent of disease can be determined with finality only through the open thorax. This attitude was a reasonable one when the risk of operative mortality was high. Two facts have caused alteration of this viewpoint. First, the risk of thoracotomy itself now approaches zero and, secondly, more pulmonary lesions are being discovered in an asymptomatic stage. Therefore, thoracotomy and excision of the lesion has become preferable to assuming the risk that a mass of unknown nature is carcinoma. At thoracotomy one does not cut into the lesion, but excises it; the "biopsy" sometimes even becomes a lobectomy. For processes not subject to diagnosis by other methods, such a course is believed wise and conservative. It is excellent treatment for the fibrocaseous tuberculous mass before cavitation has occurred. It offers the best chance for cure of really early carcinoma. It does not unnecessarily sacrifice lung tissue. In certain instances it relieves general worry about the diagnosis and the patient's future. What happier outcome could be asked than the removal of a treacherous appearing pulmonary lesion, with minimal risk of complication or fataliy, and the finding that it is a benign lesion about which there need be no further concern?

Common Sense

Thus far in the discourse I have emphasized scientific exactitude in diagnosis and discussed the various methods for its attainment. I now wish to stress common sense, not as a substitute for, but as a corollary to, the other measures.

It is admittedly desirable to pursue every tumor problem to the attainment of a morphologic answer, and toward such a conclusion at one time I unremittingly worked. With the aging process, however, has come better realization of certain facts of life. One of these is that a balance must be struck between the value to the patient of a given exact diagnosis and the benefit the patient is likely to receive from the mere fact of proof. Otherwise, patients with advanced and inoperable cancer may be submitted to tedious and expensive diagnostic procedures having little justification beyond providing laboratory proof of what is already clinically apparent, since hopeful therapy is not expected to be in any way altered thereby.

A second realization is that the so-called "only hope" type of operation, done with forethought that the cancer is advanced and beyond surgical eradication, usually proves a snare and a delusion for the patient, the surgeon, the family, and the personal physician. I wish not to be misunderstood. I favor and practice a radical and aggressive attitude in the treatment of carcinoma by surgery; but one wishes to avoid a foolish attitude. There is often strong psychological or social pressure on the family, transmitted to the surgeon directly or through the family physician, to "do everything possible," which really means "do everything helpful." Every honest surgeon naturally wishes to do this but must assume responsibility for deciding (often a lonely decision) whether the operation may be helpful or merely possible.

A third realization is that the palliative operation must really earn its name, in respite from suffering, to merit much use. Undoubtedly, this procedure has a place in the treatment of certain cases with a sequestrating lobe, copious foul sputum, and hemorrhage. But fine discrimination is required to select those whose remaining weeks or months may be made easier. To others, one may have but added to an already sad situation the burden of an operation, with its attendant pain and expensive hospitalization.

In summary of this heading entitled "Common Sense," I urge that greater diagnostic effort, intensified education of the laity, and more surgical aggressiveness be directed toward early, silent or incidentally discovered pulmonary lesions; and less of these efforts toward the late, advanced lesions.

TREATMENT

X-ray Therapy

Selectively applied X-ray therapy makes a few patients more comfortable. Widely or indiscriminately used, the average benefit fails to justify the discomfort, inconvenience, and expense of application. Its usefulness is greatest in undifferentiated, high grade epidermoid, and oat cell types. In these specified types, surgery does not alter the outlook in any demonstrable way. Pleural effusion quite often responds dramatically to X-ray therapy. An occasional patient will experience relief from severe pain. In these, the relief usually is apparent before excessive therapy has been given.

Radium offers no advantages over X-ray therapy and the facility with which

it can be applied causes X-ray therapy to be chosen in most cases to be treated by actinic radiation.

Palliative Resection

Palliative resection has been already discussed in part. It is recommended only for cases burdened with complications of secondary infection. The duration of life until death from carcinoma is in no way changed, but the patient may be prevented from dying of infection or hemorrhage and has a more comfortable terminal period.

Surgery for Eradication of Disease

At the present time complete surgical extirpation offers the only hope for cure. Pulmonary resection, including both pneumonectomy and lobectomy, in our hands now carries a 5 per cent hospital mortality risk on the basis of a 30 per cent resectability rate (1). It is my belief that lobectomy for selected upper lobe lesions and peripherally placed lower lobe lesions, particularly in older patients, is as satisfactory a procedure as pneumonectomy. Moreover, lobectomy is associated with a lower operative hazard and better subsequent functional status. For the majority of cases, however, a radical pneumonectomy is preferable.

A discussion of technical details is not considered germane to this presentation. It is assumed that the surgeon and anesthetist in charge of a given case are competent to handle such matters. It is my understanding that this audience, in respect to surgery, is more interested in what surgery should be done and why it should be done, than in exactly how it is done.

Results of Surgical Treatment

No one, however, is more interested than you in what surgery has or can accomplish. The hospital mortality from pulmonary resection for carcinoma has now been reduced to 5 per cent. Our resectability rate has been 31.8 per cent. Resected cases have a 64 per cent chance of being alive and well one year later. Rarely does an untreated case live a year after diagnosis. It was previously reported (1) that 23 per cent of the patients subjected to pulmonary resection more than five years previously were found to be living and well at the end of five years. The predominant factor affecting survival after resection was there shown to be the microscopic cell type. In this series, epidermoid carcinoma had the least gloomy prognosis, followed in sequence by adenocarcinoma, oat cell and undifferentiated types. In fact, no patient with an oat cell or undifferentiated carcinoma lived more than a year after resection. Duration of symptoms, the size of the lesion, the type of resection done and the presence or absence of involvement of lymph nodes were found to exert significant but not determinative effects upon the survival time.

OTHER LUNG TUMORS

Aside from adenoma, other varieties of lung tumor are rarities. I know of two examples of primary sarcoma and have personally treated one case of myxo-

chondroma. Unless the rare hamartoma is to be so classified, I know of no other types of lung tumor. Adenoma occurs with approximately one-tenth the frequency of pulmonary carcinoma. At least 80 per cent of the cases are found in women, and particularly young women. The rate of growth of adenoma is slow. Cough and intermittent, sporadic hemoptyses are the common symptoms and these may often be controlled for years in a particular patient by periodic bronchoscopic removal of the intralumenal portion of the tumor. The tumor, however, has a striking propensity for extralumenal growth which attains a size grossly disproportionate to the intralumenal projection. As a consequence, the gradual development of bronchiectasis is a common sequence that eventually requires lobectomy. Over 90 per cent of patients with adenoma are alive and well for five and more years subsequent to treatment. In fact, the only mortality of which I am aware among 24 cases is that of one patient who died immediately postoperatively.

Regardless of who is right in the academic argument which has proceeded for several years on the question of whether bronchial adenoma is a malignant tumor or not, one may again find comfort and security in a common sense viewpoint. The expectancy for a patient who has an adenoma removed is that she will be well and remain well thereafter. The finding of, or the failure to find, cells resembling adenoma cells in adjacent lymph nodes has, in my experience, had little, if any, bearing on prognosis. From the standpoint of the clinical course of illness, the fact is that adenoma is a different tumor entity from carcinoma and survival rate statistics concerning the two disease must be scrupulously segregated if accuracy of reporting is to be maintained.

SUMMARY

Cancer of the lung accounts for about 7 per cent of all cancers. Results of treatment are comparable to other visceral cancers. The hospital mortality rate for surgical resection is now under 5 per cent. Diagnostic methods are reviewed and evaluated. Mature judgment seasoned by common sense should be applied in the avoidance of burdensome and nonbeneficial surgery for advanced, hopeless cases. Complete surgical extirpation offers the only hope of cure at the present time.

Twenty-three per cent of cases resected more than five years were found to be living and well at the end of five years. Bronchial adenoma occurs with one-tenth the frequency of lung cancer but has at least nineteen times the survival expectancy. Clinically, it is a different entity from carcinoma, whatever its pathologic classification according to degree of malignancy may eventually prove to be.

SUMARIO

Diagnóstico y Tratamiento de los Tumores Pulmonares

Al cáncer pulmonar corresponden aproximadamente 7 por ciento de todos los cánceres. Los resultados del tratamiento se comparan a los obtenidos en otros cánceres viscerales. La mortalidad hospitalaria para la resección quirúrgica es

hoy día inferior a 5 por ciento. Después de repasar y justipreciar los métodos de diagnóstico, señálase que hay que aplicar el juicio maduro templado por el sentido común a fin de evitar la cirugía molesta y estéril en los casos avanzados desahuciados. Las únicas esperanzas de curación se cifran actualmente en la extirpación cruenta total.

Veintitrés por ciento de los casos resecados hace más de cinco años se hallaban vivos y sanos al cabo de cinco años. El adenoma bronquial no alcanza en frecuencia más que la décima parte del cáncer pulmonar, pero muestra una expectativa de sobrevivencias a lo menos diecinueve veces mayor. Clínicamente, representa una entidad distinta del carcinoma, no importa la clasificación patológica que reciba con el tiempo, conforme al grado de malignidad.

REFERENCE

(1) Adams, R.: Primary lung tumors, J. A. M. A., March 2, 1946, *130*, 547.

OBSERVATIONS ON THE CAUSES OF DYSPNEA IN CHRONIC PULMONARY GRANULOMATOSIS IN BERYLLIUM WORKERS[1]

ROBERT A. BRUCE,[2] FRANK W. LOVEJOY, JR.,[2] GEORGE B. BROTHERS[3] AND TULIO VELASQUEZ[4]

(Received for publication December 6, 1948)

INTRODUCTION

Chronic pulmonary granulomatosis in beryllium workers has been characterized by the delayed onset of weight loss, with or without anorexia, irritative coughing, exertional dyspnea, intermittent chills and fever following a known or suspected exposure by inhalation to beryllium compounds in a finely divided state (1 to 6). The most outstanding symptom has been exertional dyspnea, and there is often marked loss of weight. Clinical examination of these patients usually reveals variable degrees of respiratory distress even at rest, consisting of tachypnea, dyspnea, orthopnea and coughing. Cyanosis and clubbing of the nails may be present. Chest expansion is often limited and the vital capacity reduced. Signs of moisture may be detected by auscultation of the lungs, and the chest roentgenograms characteristically show diffuse bilateral miliary nodular shadows and reticular markings in the lung fields. Prominence of the right auricle and the pulmonary conus is noted in extreme cases. The course is one of delayed recovery in a few (7), gradual progression in many, and eventual death from right heart failure in almost a third of the patients (1, 2, 5). There is no alteration of the course by any known therapeutic agent, but supportive oxygen therapy appears to prolong life.

Preliminary respiratory studies (8) have shown that overbreathing during activity due to loss of breathing reserve and impaired pulmonary diffusion of oxygen are the earliest evidence of lesions. Arterial hypoxemia, secondary to an increased alveolar-arterial oxygen gradient, is inadequately compensated by hyperventilation especially during exertion. Advanced cases also show reduction in maximum breathing capacity together with emphysematous changes. Pathological study (1, 2, 5) of lungs from fatal cases has shown the combination of interstitial cellular infiltrations (lymphocytes, macrophages, plasma cells, and bizarre foreign body giant cells and inclusions) and diffuse patchy emphysematous alterations in alveolar structure. Right heart failure and degenerative changes in the liver have also been observed. Recovery of beryllium in most of the or-

[1] This work is part of a cooperative study of beryllium toxicology in human subjects carried out by the Department of Medicine of The University of Rochester School of Medicine and Dentistry under the direction of Dr. William S. McCann in collaboration with the Division of Medical Services of the Atomic Energy Project and aided by grants from the Lovejoy and Hochstetter Funds, University of Rochester School of Medicine and Dentistry.

[2] Bertha Hochstetter Buswell Research Fellow in Medicine.

[3] Fellow of the General Education Board.

[4] Rockefeller Visiting Fellow in Medicine from Lima, Peru.

gans of a fatal case has completed the evidence for the specific etiology of granulomatosis in human subjects (9).

Observations on the cardio-respiratory functions[5] of 6 patients[6] with chronic delayed pulmonary granulomatosis due to beryllium are reported here (table 1 for physical data). The cases are arranged in order of increasing severity of disease from borderline disturbances to total disability. Four of these patients had known contacts with beryllium compounds under industrial working conditions. The other 2 patients were "neighborhood cases" since they did not work in the industry, but had contact with beryllium compounds only from atmospheric contamination in the close proximity to a manufacturing plant. The duration of respiratory distress in these 6 patients ranged between one and six years. The etiology was presumed in most by exclusion of all other differential diagnoses

TABLE 1

Physical data on patients studied

PATIENT	AGE	SEX	HEIGHT	WEIGHT	S. AREA	AP DIAMETER	RADIOLOGICAL CHEST VOLUME	TRANSVERSE CARDIAC RATIO
			cm.	*Kg.*	*sq. m.*	*cm.*	*liters*	
A. B.	26	M	169	56.0	1.65	17.5	9.22	0.446
M. C.	40	F	157	58.3	1.57	21.0	15.85	0.470
M. E. T.	23	F	167	59.9	1.66	20.0	11.14	0.384
M. H. C.	28	F	165	52.6	1.54	18.7	7.97	0.422
J. J.	31	M	162	52.8	1.54	21.0	8.83	0.524
W. R.	35	M	183	51.8	1.68	24.0	15.24	0.464

and was established in the third patient by identification of trace amounts of beryllium in the urine. In the sixth patient beryllium was recovered from kidney stones.[7]

METHODS

These patients were hospitalized for periods of observation ranging between two weeks and three months under complete metabolic control at all times. Except where indicated, all the measurements reported were obtained during nonfasting conditions. *Maximum breathing capacity* was measured by means of voluntary maximal hyperventilation for 30 seconds with the patient standing, using a mouth piece, high velocity valve, and a Douglas bag. The gas volume was measured in a standard Tissot spirometer, corrected to 37° C. and recorded per square meter of body surface area. Predicted values for both maximum breathing capacity and lung volumes were based on regression formulae derived by the investigators in the Chest Service of Bellevue Hospital (10). *Recumbent lung volumes* were determined in duplicate by means of the helium dilution technique (11). All partitions of the total capacity were corrected to 37° C. *Dead space* measurements were obtained and corrected to 37° C. by breathing data obtained from a modified A-13 Air Force face mask

[5] The metabolic studies on these patients will be the subject of a separate report.

[6] We are indebted to Dr. Joseph DeNardi of Lorain, Ohio, and Dr. Gordon Richardson of Towanda, Pennsylvania, for aiding in the arrangements in studying these patients.

[7] We are indebted to Dr. L. T. Steadman of the Department of Radiation Biology for making these measurements.

and continuous gas analyzer (12). *Bronchospirometry* (13) was performed with patients breathing oxygen and, when feasible, leg exercises were carried out during part of this procedure. Measurements of *alveolar pressure-velocity* relationships were obtained[8]. *Blood gas data* were based on arterial and mixed venous blood samples. Syringes containing 5 per cent sodium fluoride in heparin were routinely employed. Under sterile 1 per cent procaine infiltrative anesthesia, blood was obtained from either radial or femoral artery, and by means of *catheterization* of the right heart by Cournand's technique (14). Attempts were made to obtain respiratory data simultaneously for calculations of alveolar arterial gradients (employing Riley's method of calculating alveolar pO_2) (15) as well as cardiac output by direct Fick principle. The effects of mild exercise with the legs and of breathing pure oxygen were also noted. In one patient, who developed congestive heart failure, the initial effect of intravenous *digoxin* therapy were observed during a second catheterization, and in another the initial effects of intravenous pyrogen-free cytochrome C[9] were studied. The alterations of performance and of blood gas content from the ingestion of either *sodium chloride* or *ammonium chloride* were investigated in selected patients. Blood oxygen and carbon dioxide contents and capacities were measured by the Van Slyke (16) and Roughton-Scholander techniques (17), and gas tensions by the micro method of Riley, *et al.* (15).

In additon to the above, cardiac studies included serial *electrocardiograms* with complete chest leads (CF 1–6), *ballistocardiograms* (18), *pneumocardiograms* (19) and *intracardiac pressure-pulse* recordings employing a Statham Pressure Transmitter (20). *Venous pressure* was measured with a saline manometer, using 5 cm. below the sternum during recumbency as the zero reference point. *Circulation times* were measured simultaneously with a mixture of two drops of U.S.P. ether for arm-to-lung time, and 2 ml. of Macosol for arm-to-mouth time.

Exercise studies (21) were performed according to standardized routine of 10 minute periods of *rest*, *walking* on a motor-driven treadmill, and *recovery*. The rate of walking was either moderate 2.6 mph, or slow 1.7 mph (8 and 12 rpm or 46.7 and 70 meters per minute, respectively) and the grade of incline was either 0 per cent, 5 per cent, or 10 per cent. Simultaneous measurements at minute intervals were made of the heart rate, blood pressure, chest lead electrocardiogram, ventilation volume, respiratory rate, expired and "mid-capacity"[10] (rather than "alveolar") oxygen tension and carbon dioxide concentration. From these primary data, ventilation indices, oxygen consumption and debt, respiratory quotients, dead space volumes, etcetera, were calculated. All these gas volume data were corrected to square meter of body surface area at 0°C., 760 mm. mercury dry gas for ease of comparison between individuals. The appearance of symptoms of distress were recorded in relation to the other data. In 2 patients and one control, repeated exercise studies (as many as 32) were made to ascertain the range of variability in the several measurements listed above during exercise.

RESULTS

Patients with mild symptoms may show almost no physical signs of disease, yet exhibit characteristic changes on roentgenography of the chest. Functional studies reveal appreciable deviations from normal performance that account for the exertional dyspnea and intolerance of more than moderate activity.

[8] We are indebted to Dr. Arthur Otis, Department of Physiology, for making these measurements.

[9] Obtained from Biorganic Laboratories, Inc.

[10] In the traditional sense, measurement of alveolar air involves determination of gas concentrations after *forced* expiration; "mid-capacity" concentrations refer to those obtained at the end of normal expiration. In exercise studies, only the "mid-capacity" can be used.

Cases

A. B., a 27 year old white married male, had industrial contact with irritating fumes from a beryllium alloy process for three months during 1940. He was in excellent health until 1944, when he developed bouts of chills, fever, irritative coughing, wheezing and dyspnea on exertion while in the Army. Chest roentgenographic examination was reported negative at that time, but by October, 1947, symptoms had progressed, weight loss from anorexia had occurred, and another chest roentgenogram revealed diffuse bilateral lesions compatible with beryllium granulomatosis. He has been unable to work since that time. On examination in March, 1948, vital signs were normal except for slight tachypnea. The patient appeared slender; he was using his accessory muscles of respiration even for quiet breathing. There was neither cyanosis nor clubbing of the fingers and the chest examination was otherwise unremarkable except for accentuation of breath sounds over the left lower lobe of the lung. A roentgenogram of the chest showed enlarged hilar root shadows with soft outlines and diffuse nodularity throughout the lung fields (figure 1a). The patient was unable to remain for complete studies, but the following observations were made. The red blood count was 5,600,000 per cu. mm., the hemoglobin concentration was 22.3 Gm. per 100 cc. and the hematocrit was 51 per cent. The respiratory pattern was irregular, with sighing indicative of emotional disturbance, which was compatible with the observed behavior. His maximum breathing capacity was only 56 per cent of the predicted value and in some measure was low because of poor cooperation. Determination of lung volumes showed marked reduction of total capacity (table 2, figure 2) to 3.0 liters, or 58 per cent of the value predicted from the height formula. Measurement of chest volume on the roentgenogram was also reduced, suggesting inability to adequately expand the chest, and the vital capacity was limited to 2.0 liters recumbent, or 2.5 liters standing. The alveolar pressure-velocity measurements were normal (table 2). The electrocardiogram, the ballistocardiogram, the venous pressure, and circulation times, were all normal (table 3). The patient was able to perform standard exercise tests (at rate of 2.6 mph) at inclines of 0, 5, and 10 per cent grades for 10 minutes (table 4, figure 5). On the latter two, he experienced severe dyspnea, sweating and substernal distress. His legs became very tired. There was a paradoxical pulse with average heart rates up to 134 per minute. No electrocardiographic changes were observed, and the oxygen debts were normal. The ventilation indices were but slightly increased, and the pulmonary efficiency in relation to work was almost normal (figure 3).

This patient's reduced ventilatory capacity and total lung volume were in contrast to his apparent inability to hyperventilate during rest or exercise as much as other patients (figure 3). Possibly the secondary polycythemia accounted for this. Unfortunately no arterial blood samples were obtained in order to evaluate either the alveolar oxygen gradient or the degree of hypoxemia. In an older individual, relatively the same degree of granulomatous change may be associated with greater functional impairment, as indicated by the next case.

M. C., a 40 year old white woman, developed a contact dermatitis on both arms and hands as a result of an industrial exposure to beryllium powders for three months during 1944. She discontinued this work and was in good health until August, 1946, when she began to notice coughing while on vacation in Oregon. Exertional dyspnea was first noted in June, 1947; a chest roentgenogram obtained a few months later revealed "cloudy" changes compatible with "beryllium poisoning." She experienced no anorexia, and weight increased from 128 to 136 pounds during the last six months of 1947. On examination in April, 1948, vital signs were normal, and the patient appeared well. Frequently she exhibited an irritative coughing, put showed no tachypnea, dyspnea, cyanosis, limitation of chest expansion, or clubbing of the fingers. Occasionally wheezes and rhonchi were audible on

auscultation of the lungs. The heart was not enlarged, the rhythm was regular, and the sounds were of good quality, although the second pulmonic sound was accentuated. Roentgenographic examination of the chest showed moderately fine, confluent, soft nodulations scattered diffusely through both lung fields (figure 1b). (Metabolic studies revealed no abnormalities except for slight lowering of plasma albumin to 3.3 Gm. per 100 cc. as determined by electrophoresis. Because of a tendency towards rouleaux formation, the red blood cell count was 6.76 millions per cu. mm., but the contentration of hemoglobin was only 11.8 Gm. per 100 cc.) The maximum breathing capacity was 97 per cent of the expected

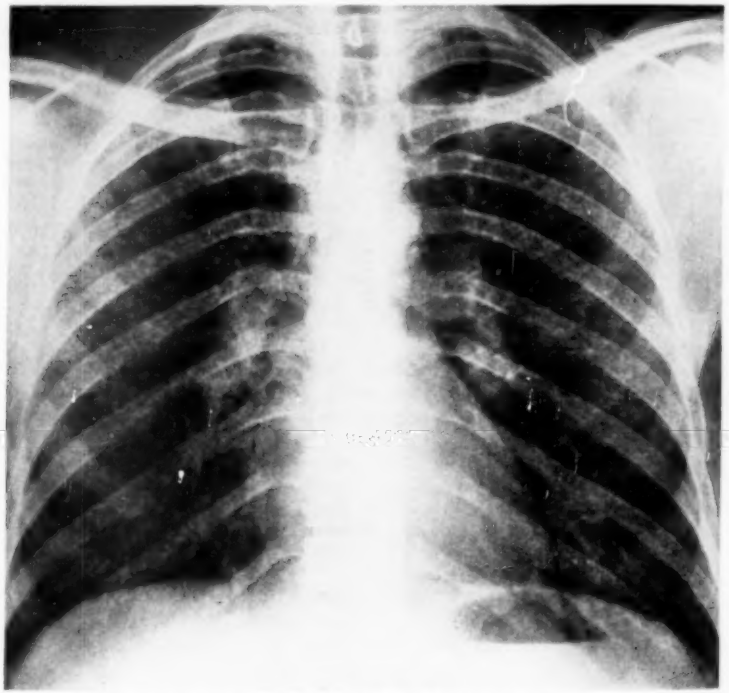


FIG. 1a. Chest roentgenogram of patient A. B. with chronic pulmonary granulomatosis associated with beryllium compounds.

values (table 2), and the breath-holding time was 34 seconds. The vital capacity and total pulmonary capacity were reduced to 1.87 and 3.21 liters, respectively, or 67 per cent and 89 per cent of the predicted values (table 2, figure 2).

Bronchospirometry while breathing oxygen showed that the proportion of ventilation between the two lungs was normal, but that 67 per cent of the oxygen uptake was performed by the right lung. The electrocardiogram was normal, as were the venous pressure and the arm-to-mouth circulation time, but the arm-to-lung circulation time was prolonged to 10 seconds (table 3). By right heart catheterization, the right ventricular pulse pressure was 25 to 37 mm. of mercury during rest, and increased to 45 to 67 mm. of mercury during mild leg exercises. The resting cardiac output was normal: 4.7 liters per minute by direct Fick principle, and increased to 6.65 liters during exercise. Blood gas studies showed a resting

arterial oxygen saturation of 86 per cent, hemoglobin concentration of 12.46 Gm. per 100 cc., and pO_2 of 75 mm. The alveolar-arterial oxygen gradient was not greatly increased, and the resting level of ventilation was a high normal value despite the presence of borderline anemia and definite hypoxemia (figure 4). Exercise studies showed that the patient was able to walk (at the rate of 2.6 mph) on the level without symptoms, experienced dyspnea at 5 per cent grade of incline, and was unable to walk more than five minutes at 10 per cent grade because of more intense dyspnea. The ventilation indices were moderately increased whereas the oxygen debts were normal at 0 and 5 per cent grades, but were in-

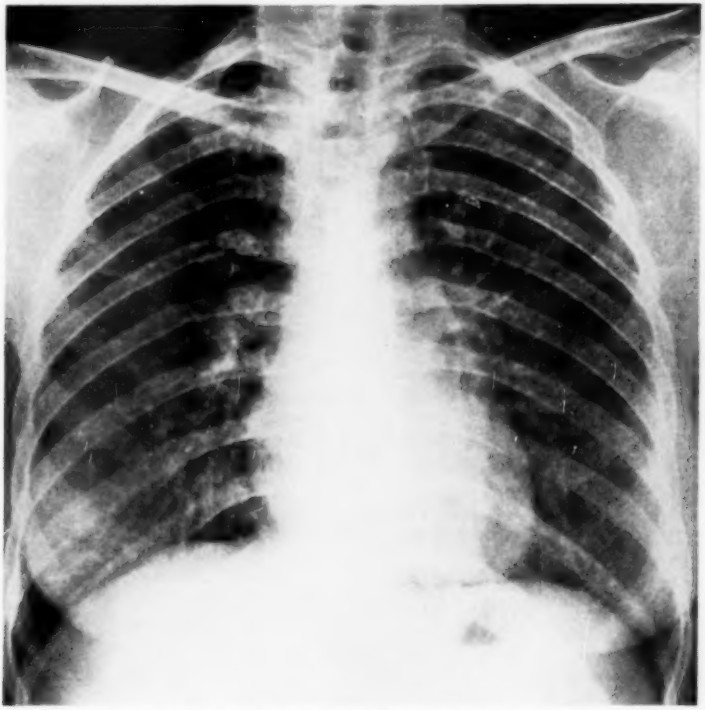


FIG. 1b. Chest roentgenogram of patient M. C. with chronic pulmonary granulomatosis associated with beryllium compounds.

creased markedly to 54 per cent at 10 per cent grade walking (table 4). The pulmonary efficiency in relation to work (figure 3) was appreciably reduced.

This patient, M. C., also had moderate reduction in vital capacity, yet in contrast to A. B. was able to maintain a normal maximum breathing capacity. Her pulmonary efficiency was impaired because of moderate increase in alveolar-arterial gradient which prevented normal saturation of arterial blood with oxygen. With increased arm-to-lung circulation time and pulmonary hypertension during exercise, she presented evidence of pulmonary resistance and right heart strain (noted by accentuation of the second pulmonic sound). Under

ordinary activity the exertional dyspnea was due to pulmonary insufficiency only, whereas with more strenuous exertion the unusually large oxygen debt indicated acute circulatory insufficiency and possible incipient heart failure.

Another of these patients experienced attacks of dizziness in addition to exertional dyspnea as an aftermath of diffuse pneumonitis.

M. E. T., a 23 year old white woman, had intermittent exposure to atmospheric contamination with dust containing beryllium compounds for an indefinite period during pregnancy

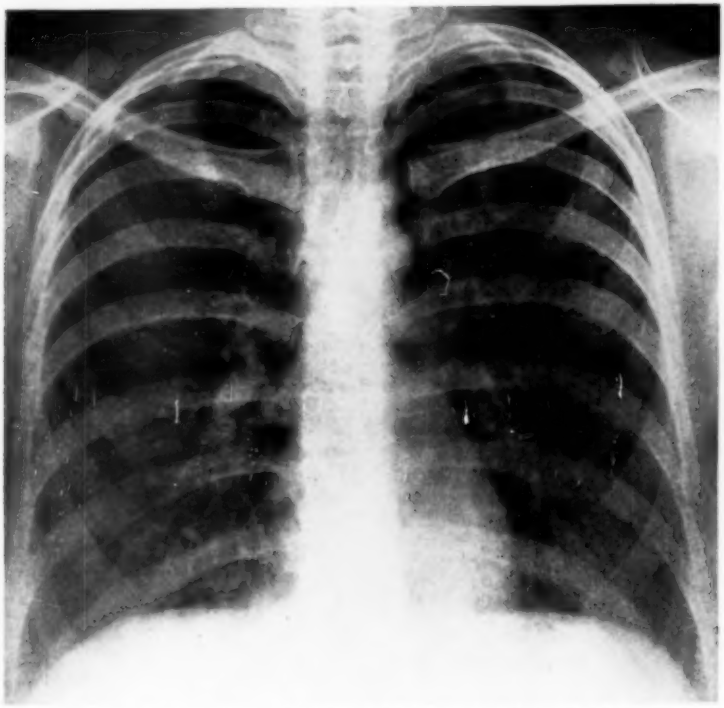


FIG. 1c. Chest roentgenogram of patient M. E. T. with chronic pulmonary granulomatosis associated with beryllium compounds.

in 1946. Following this, the patient lived with her parents adjacent to a plant manufacturing beryllium products. Fumes from this factory caused pharyngeal irritation and coughing, especially on "damp and muggy days." By March, 1947, she noted exertional dyspnea, coughing productive of mucous, substernal tightness in chest, and occasionally nausea. Later when she developed chills, fever and roentgenographic evidence suggestive of miliary tuberculosis, she was hospitalized in a sanatorium. She exhibited a febrile course, required continuous oxygen therapy for dyspnea and cyanosis, and was treated with both penicillin and streptomycin for a few days. She gradually recovered, became ambulatory, and was observed here from December, 1947, to February, 1948. On admission to Strong Memorial Hospital she appeared almost well, but there was cyanosis of the nails. Chest expansion

was limited and inconstant rales were audible over the base of each lung. Roentgenographic examination of the chest showed diffuse pulmonary haziness, reticular markings and minute nodulations; the heart was not enlarged (figure 1c). With exertion the patient became dyspneic and complained of dizziness. During the period of observation the intensity of these two symptoms fluctuated considerably, and at times she became easily fatigued and exhibited orthopnea. Some of the variation in symptomatology was related to emotional factors represented by frequent sighing respirations and a tendency to hyperventilate. The routine hospital laboratory data were normal, except for reduction in plasma albumin concentration to 3.3 Gm. per 100 cc., as determined by electrophoresis, and diminished blood volume by the Evans blue technique[11]. The patient's predicted blood

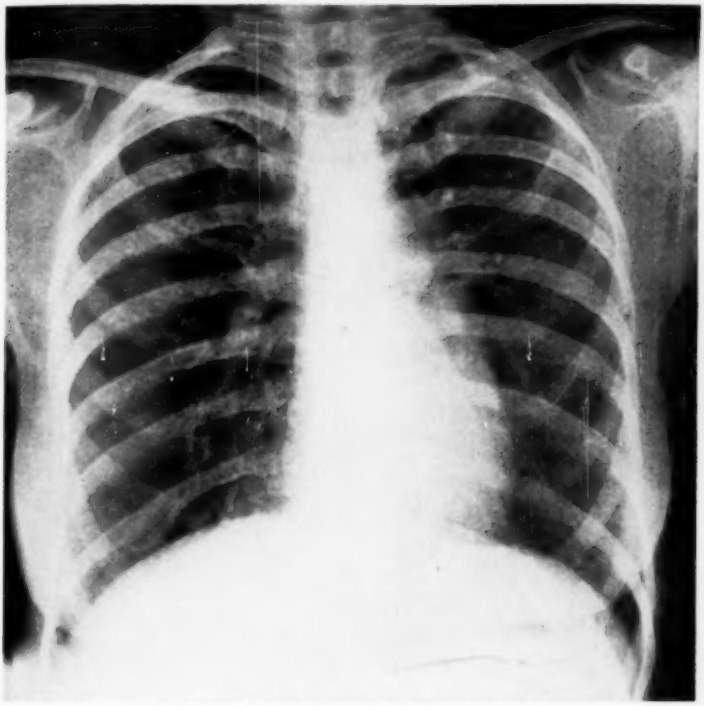


FIG. 1d. Chest roentgenogram of patient M. H. C. with chronic pulmonary granulomatosis associated with beryllium compounds.

volume was 4,050 ml., but the observed value was 2,960 ml. with an hematocrit of 44.5 per cent and plasma volume of 1,650 ml. The maximum breathing capacity was 53 per cent of the predicted value (table 2). Alveolar pressure-velocity relationships were normal. Determination of lung volumes showed a moderate reduction of both the total pulmonary capacity and the vital capacity (table 2, figure 2). In an attempt to evaluate further the dyspnea and dizziness by altering her electrolyte balance, the patient was given a course of sodium chloride 2.0 Gm. orally, four times daily, for four days prior to the expected onset of

[11] We are indebted to Dr. L. A. Kohn for these data.

menses. Serial measurements of lung volumes showed that the total and vital capacities diminished appreciably. She complained of greater exertional dyspnea and orthopnea, together with diminished exercise tolerance. The venous pressure was 11 cm. of water, and rose to 12 cm. with pressure over the right upper quadrant of the abdomen. There was no significant change in basal weight from day to day. The observed blood volume increased to 3,600 ml. with an hematocrit of 41.8 per cent and a plasma volume of 2,100 ml. Coincident with the onset of the menstruation, the patient was then given 2.0 Gm. of ammonium chloride orally, three times daily, instead of salt. The basal weight declined 1.0 Kg., the exertional dyspnea diminished, but the intensity of dizziness became so severe that the ammonium chloride had to be discontinued after forty-eight hours. Concomitantly there

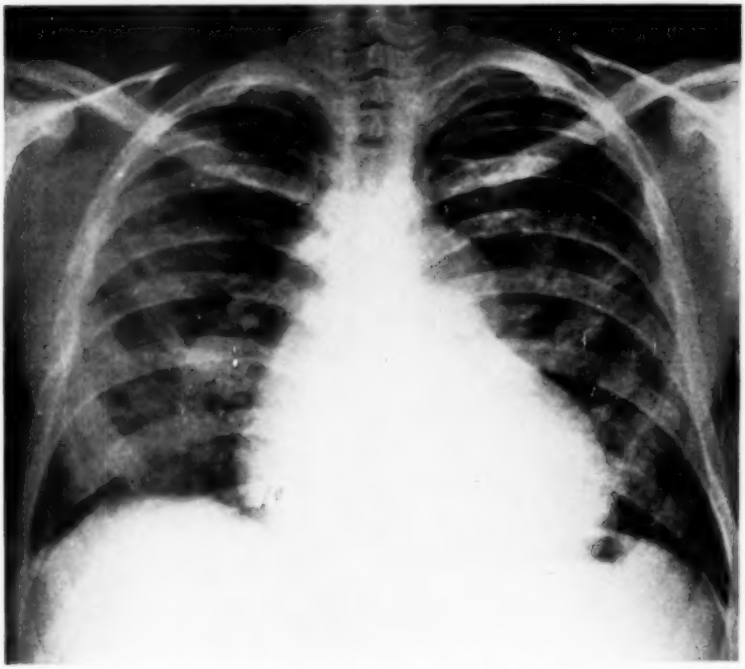


FIG. 1c. Chest roentgenogram of patient J. J. with chronic pulmonary granulomatosis associated with beryllium compounds.

was a reduction in the arterial pCO_2 from 44.5 mm. to 36.5 mm., and the residual air volume declined to 410 ml. or 13.5 per cent of the total capacity. The arm-to-lung circulation time was prolonged to 14 seconds. The performance of exercise caused intense substernal pain during this period, although no electrocardiographic alterations were observed. Less marked changes were observed when the ammonium chloride was repeated one week later, after the completion of the menses.

Right heart catheterization showed a normal resting right ventricular pulse pressure of 16 mm. of mercury. The arterial blood was 87 per cent saturated with oxygen and the pO_2 was 72 mm. Repeated exercise tolerance studies at 0, 5, and 10 per cent grades of incline showed considerable daily fluctuation in intensity of dyspnea and dizziness, as well as endurance. Patient was able to walk only seven minutes at 5 per cent grade, and four

minutes at 10 per cent grade because of dyspnea and dizziness. Although the ventilation indices were increased, the oxygen debts were normal, except for the 10 per cent grade when it was 22 per cent, largely because of the shortened exercise period. In a series of exercise tests at 0 per cent grade, analysis of the coefficients of variability of the several measure-

FIG. 1f. Chest roentgenogram of patient W. R. with chronic pulmonary granulomatosis associated with beryllium compounds.

ments obtained during exercise showed the oxygen consumption to be the most variable (5.7 per cent) whereas the rate of oxygen absorption per liter of air ventilated was the least variable (2.4 per cent). Differences in anxiety, rather than in gaseous diffusion, therefore, seemed to account for the variations in the patient's symptoms from day to day.

This patient's exertional dyspnea also was related to reduction in ventilatory capacity and complementary lung volume, in addition to the hyperventilation

compensating for hypoxemia. There was no evidence of right ventricular strain under ordinary circumstances. The dizziness may have been in part related to

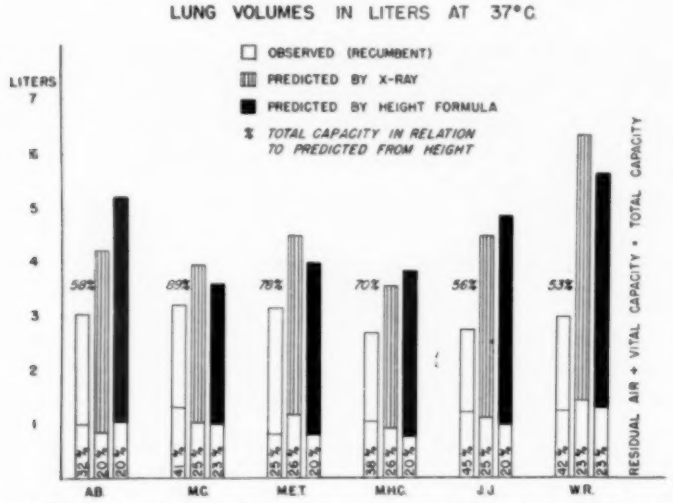


FIG. 2. Relationship of observed pulmonary volumes of patients to predicted values based on height and age, and chest roentgenograms.

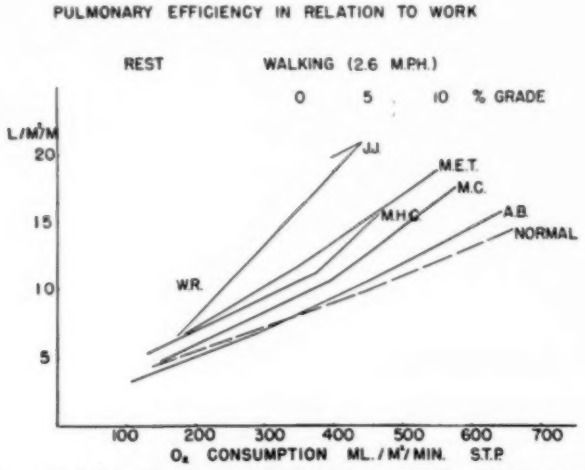


Fig. 3. Pulmonary efficiency in relation to work performed while walking on the treadmill.

hypocapnia, for the ingestion of ammonium chloride during the menstrual period was associated with a reduction in arterial pCO_2. Since the residual air volume simultaneously was markedly reduced, the buffering action of this lung volume

partition may have been sufficiently altered to permit greater escape of carbon dioxide without significant increases in ventilation. The mechanism of this reversible action is difficult to explain, since it was less marked when repeated after menstruation, but may have been related to pulmonary venous vasoconstriction. Finally, the measurement which reflected the diffusion of oxygen into the blood during exercise was the least variable value, and showed no correlation with the patient's variations in symptoms. The fourth patient in this series

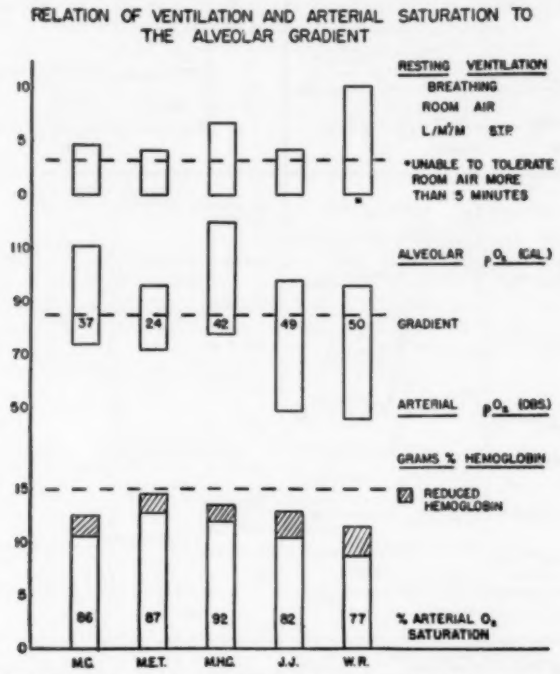
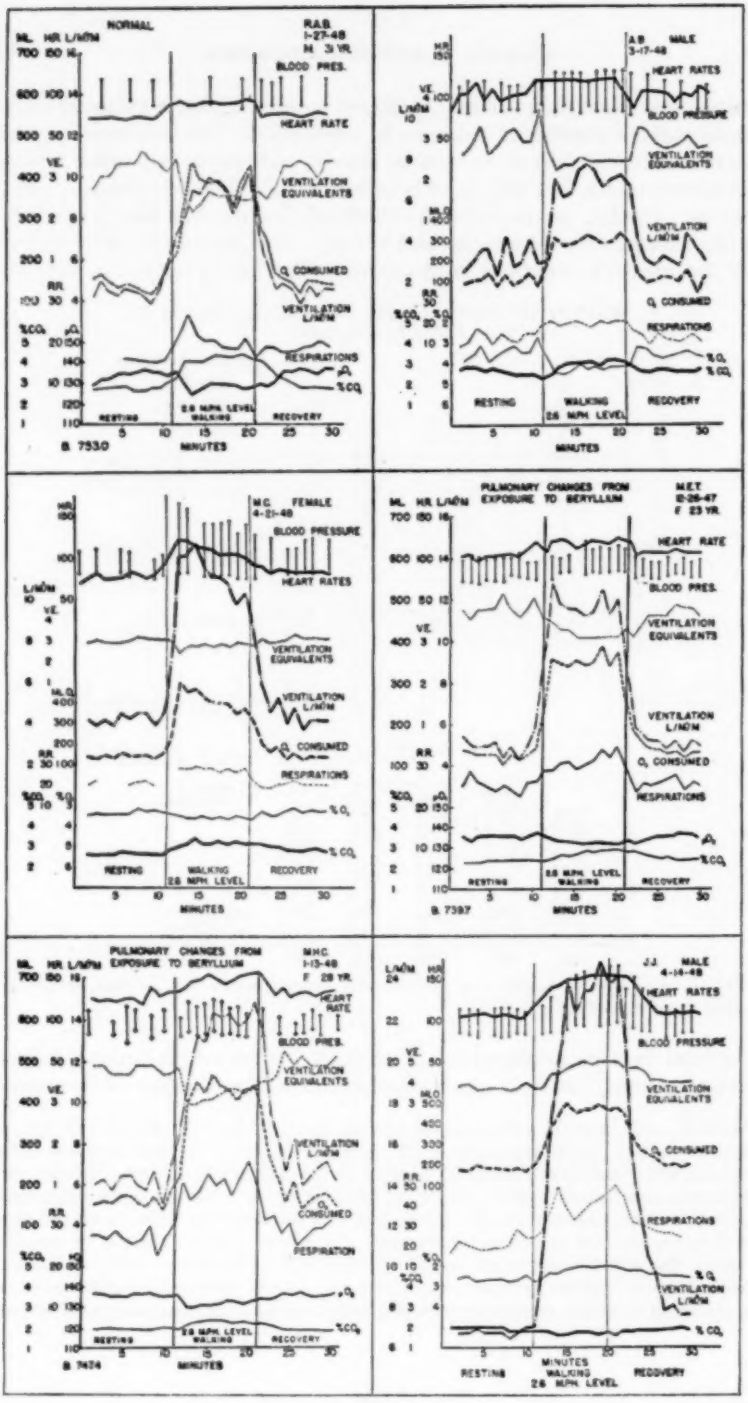


FIG. 4. Relation of ventilation and arterial oxygen saturation, at rest, to alveolar-arterial oxygen gradient.

came from the same neighborhood as the third, but her manifestations of chronic pulmonary granulomatosis were somewhat different at the time of observation.

M. H. C., a 28 year old white married woman, also lived in close proximity to the same beryllium factory (as did patient M. E. T.) from 1944 to 1948. In May, 1946, she noticed coughing which was occasionally productive of blood-streaked sputum. Inhalation of fumes from this factory were known to be irritating to her. The paroxysms of coughing gradually became more severe, and by July, 1947, she had recurrent chills, fever, orthopnea, and dyspnea at night as well as on exertion. Because of anorexia she lost 28 pounds in weight. Cyanosis became prominent on exertion, and in November, 1947, she moved to another location and her symptoms began to subside. On examination in January, 1948, she exhibited moderate respiratory distress, limitation of chest expansion, mild cyanosis,


NORMAL
R.A.B.
1-27-48
M 31 YR.
BLOOD PRES.
HEART RATE
VENTILATION EQUIVALENTS
O2 CONSUMED
VENTILATION L/MIN
RESPIRATIONS
RESTING
2.5 MPH. LEVEL WALKING
RECOVERY
B. 7530
MINUTES
A.B.
3-17-48
MALE
HEART RATES
BLOOD PRESSURE
VENTILATION EQUIVALENTS
VENTILATION L/MIN
O2 CONSUMED
RESPIRATIONS
RESTING
WALKING 2.5 MPH. LEVEL
RECOVERY
MINUTES
W.G.
4-21-48
FEMALE
BLOOD PRESSURE
HEART RATES
VENTILATION EQUIVALENTS
VENTILATION L/MIN
O2 CONSUMED
RESPIRATIONS
RESTING
WALKING 2.5 MPH. LEVEL
RECOVERY
MINUTES
PULMONARY CHANGES FROM EXPOSURE TO BERYLLIUM
M.E.T.
12-29-47
F 23 YR.
HEART RATE
BLOOD PRES.
VENTILATION EQUIVALENTS
VENTILATION L/MIN
O2 CONSUMED
RESPIRATIONS
RESTING
2.5 MPH. LEVEL WALKING
RECOVERY
B. 7387
MINUTES
PULMONARY CHANGES FROM EXPOSURE TO BERYLLIUM
M.H.C.
1-13-48
F 28 YR.
HEART RATE
BLOOD PRES.
VENTILATION EQUIVALENTS
VENTILATION L/MIN
O2 CONSUMED
RESPIRATION
RESTING
2.5 MPH. LEVEL WALKING
RECOVERY
B. 7474
MINUTES
J.J.
4-14-48
MALE
HEART RATES
BLOOD PRESSURE
VENTILATION EQUIVALENTS
O2 CONSUMED
RESPIRATIONS
VENTILATION L/MIN
RESTING
MINUTES WALKING 2.5 MPH. LEVEL
RECOVERY

and slight clubbing of the fingers. There was a sinus tachycardia, averaging around 120 per minute, and the blood pressure was reduced to 100/70 mm. of mercury. Rales were audible over the lung bases. Roentgenographic examination of the chest showed diffuse granular infiltrations throughout the lung fields (figure 1d). The maximum breathing capacity was 43.5 liters per square meter, or 75 per cent of the predicted value (table 2). Breath-holding time was 23 seconds. Alveolar pressure-velocity relationships were normal.

TABLE 2

Maximum breathing capacity, lung volumes, and physiological dead spaces in liters at 37° C. and alveolar pressure at 500 ml/sec. velocity air flow

PATIENT	MAXIMUM BREATHING CAPACITY			VITAL CAPACITY	COMPLEMENTARY AIR	RESERVE AIR	MID-CAPACITY	RESIDUAL AIR	TOTAL CAPACITY	M.C.	R.A.	TIDAL	DEAD SPACE	ALVEOLAR* PRESSURE
	Obs.	Pred.	Per cent											
										per cent	*per cent*			*mm.*
A. B.	41.0	73.0	56	2.05	1.11	0.71	1.69	0.98	3.03	56	32	0.62	—	13.5
M. C.	50.7	52.4	97	1.87	1.19	0.65	1.99	1.34	3.21	62	41	0.51	—	—
M. E. T.	31.9	60.4	53	2.34	1.46	0.52	1.31	0.79	3.13	42	25	0.50	0.161	12.0
M. H. C.	43.5	58.0	75	1.67	0.71	0.51	1.54	1.03	2.70	57	38	0.67	0.155	12.0
J. J.	51.5	70.0	74	1.51	1.27	0.76	1.46	1.23	2.74	53	45	0.47	0.120	—
W. R.	10.2	68.0	15	1.74	1.60	0.78	2.04	1.26	3.00	67	42	0.86	—	9.0

* Mean normal pressure in 30 normals is 11.3 mm. ± 3.43[8].

TABLE 3

Cardiovascular measurements at rest

PATIENT	VENOUS PRESSURE, CM. OF WATER	CIRCULATION TIME		RIGHT AURICULAR PRESSURE	RIGHT VENTRICULAR PRESSURE	CARDIAC OUTPUT	STROKE VOLUME	BALLISTOCARDIOGRAM
		A-L	A-M					
		sec.	*sec.*			*L/min.*	*ml.*	
A. B.	9.4	7	15	—	—	—	—	Normal
M. C.	8.4	10	15	5	25	4.7	58	Borderline
M. E. T.	9.5	8.8	15	—	12	—	—	Low amplitude
M. H. C.	8.1	7	14	—	25	—	—	Shallow I, deep K Low amplitude
J. J.	13.5	11	16	13	78	3.09	28	Borderline normal
W. R.	−1.0	7.5	14	30	65	5.92*	70	Abnormal (marked respiratory variations

* Breathing oxygen.

Determination of lung volumes revealed that the vital capacity was reduced to 1.67 liters or 54 per cent of the predicted value (figure 2), and a less marked reduction in total capacity was

FIG. 5. Exercise performance of one normal control (R. A. B.) and 5 patients in order of increasing cardio-respiratory disability.

Top row, left to right: R. A. B. (control) and patient A. B.; Second row, left to right: M. C. and M. E. T.; and Bottom row, left to right: M. H. C. and J. J. (Note paradoxical responses in J. J.).

observed. Except for sinus tachycardia and depression of the ST segment in lead 1, the electrocardiogram was unremarkable. Venous pressure, circulation times, and blood pressure were normal, but the right ventricular pulse pressure at rest was near the upper limits of normal (table 3). Despite the low vital capacity the arterial blood was 92 per cent satu-

TABLE 4

Exercise responses to treadmill walking at 2.6 mph

PATIENT	ENDURANCE	VENTILATORY EQUIVALENT*		VENTILATION INDEX**	OXYGEN DEBT	HEART RATE*	BLOOD PRESSURE*	VENTILATION*	O_2 CONSUMPTION	R.Q.*	SYMPTOMS AND SIGNS
		Rest	Exercise								
	min.				*per cent*		*mm.Hg*	*L./M²/min.*	*ml./M²/min.*		
0 per cent Incline											
A. B.	10	3.00	2.38	8.16	7	120	130/81	6.82	286	0.71	Slight dyspnea
M. C.	10	2.99	2.75	10.3	9	110	145/77	10.81	395	0.83	None
M. E. T.	10	3.90	3.34	13.8	10	125	118/87	11.78	352	0.92	Slight dyspnea
M. H. C.	10	3.80	3.01	19.8	15	143	129/85	11.35	376	—	Dyspnea, fatigue
J. J.	9	3.79	4.72	38.2	30	108	117/83	20.96	439	0.83	Severe dyspnea, cough, fatigue
5 per cent Incline											
A. B.	10	3.00	2.36	14.0	16	124	131/79	10.64	451	0.83	Severe dyspnea, sweating, substernal distress
M. C.	10	3.06	2.95	16.5	16	131	—	14.67	506	—	Dyspnea, fatigue
M. E. T.	7	3.50	3.40	16.8	9	133	—	15.13	445	0.78	No comments
M. H. C.	7	4.40	3.33	21.5	16	150	140/77	15.40	464	—	Dyspnea, left chest pain
J. J. (1.7 mph).	10	4.48	4.88	36.2	27	141	116/74	19.65	398	1.07	Low voltage ECG
10 per cent Incline											
A. B.	10	3.20	2.47	16.5	12	134	154/81	15.89	641	0.83	Severe dyspnea, sweating, legs tired, paradoxical pulse
M. C.	5.16	3.08	3.07	25.0	54	148	161/76	17.60	574	0.98	Dyspnea, sweating
M. E. T.	4.5	3.50	3.31	20.3	22	136	117/71	18.15	548	0.93	Dyspnea, dizziness

* Average value per minute for exercise period.

** Mean ventilation index in 35 normals is 6.12 ± 2.08.

rated, and the pO_2 was 78 mm. Exercise tolerance studies demon strated that she was able to walk at 2.6 mph and at 0 and 5 per cent grades, but that fatigue and dyspnea developed even with the least exertion. Her tolerance was shortened by grade walking to seven minutes, and she was unable to walk at 10 per cent grade without supplemental oxygen. The ventilation indices were greatly elevated to 19.8 and 21.5 at 0 and 5 per cent grades, re-

spectively, showing the extent of the hyperventilation which prevented more serious degrees of hypoxemia (table 5). Pulmonary efficiency in relation to work diminished as soon as grade walking was attempted (figure 3). The least variable factor during exercise, based upon coefficients of variability of multiple tests, was the rate of oxygen absorption per liter of ventilation (5.3 per cent), whereas ventilation volume exhibited the greatest variation (11 per cent). The oxygen debts were normal, yet clinically the patient often exhibited marked accentuation of second pulmonic sound, transient gallop rhythm, left chest pain, sweating, and exhaustion as a result of this exertion. She presented no signs of heart failure, and the administration of 1.5 mg. of digoxin orally in two divided doses caused anorexia and nausea but failed to diminish intensity of exertional dyspnea or to improve exercise tolerance or performance. Ammonium chloride 2 Gm. orally, three times daily, for two days induced anorexia and dizziness, but no other changes similar to those in patient M. E. T. were observed.

This patient, M. H. C., differed from the preceding one by more intense exertional dyspnea because of a smaller vital capacity. Her maximum breathing capacity was appreciably greater, and this may account for the more nearly normal level of arterial oxygen saturation. During the period of observation there was an appreciable increase in vital capacity together with a slowing of the heart rate at rest and exercise. The exercise tolerance was not as good, however, probably because of pulmonary hypertension during exertion. In contrast to this patient is the disability from advanced cor pulmonale exhibited by the fifth and sixth patients, both of whom had definite industrial exposures to beryllium compounds.

J. J., 31 years of age, worked for two months as a sifter of fluorescent powders containing beryllium. Despite protective clothing and face mask, he had to discontinue this work in December, 1942, because of coughing and exertional dyspnea. Roentgenographic examination of the chest was reported to show "dust in the lungs." After these symptoms subsided he returned to work on another job which exposed him to fumes of various acids and ammonia, as well as beryllium, tungsten, and carborundum dusts on various occasions. Early in 1945, he had a recurrence of coughing, exertional dyspnea, together with fatigue, anorexia, weight loss, night sweats, chills and fever which progressed over three months. Paroxysmal nocturnal dyspnea and orthopnea were also noted. He was admitted to Trudeau Sanatorium where exhaustive diagnostic and functional studies[12] were made. The arterial oxygen saturation was 86 per cent and the total lung volume was 2.73 liters with 24 per cent residual air content. The exercise tolerance was limited to 2.5 mph level walking for 5 minutes, and the ventilatory equivalent for oxygen was 4.7 liters per 100 ml. of oxygen per minute. During the next year and one-half the patient improved somewhat, but lost 40 pounds in weight. Tubercle bacilli were never demonstrated in the sputum and a tuberculin skin test was negative to 10 mg. Examination of material obtained from biopsy of skin lesions on the hand showed lesions with noncaseous central necrosis which were interpreted as "changes characteristic of Boeck's sarcoid." Minimal symptomatic relief was obtained from quinine and neoarsphenamine therapy. When the patient was returned home, he gradually improved, gained weight from 117 to 133 pounds and by the summer of 1947 was able to mow part of his lawn. After that time, clubbing of the fingers, accentuation of the second pulmonic heart sound, more exertional dyspnea, cyanosis, and weight loss indicated further hypoxemia and cor pulmonale. Examination in April, 1948, revealed a chronically ill, malnourished white male, with obvious respiratory distress even at rest. There was tachypnea of 24 per minute at rest, cyanosis, marked clubbing of the nails, limited chest expansion and

[12] Functional studies were done by Dr. George Wright, Saranac Lake, New York.

utilization of the accessory muscles for respiration. The lungs were resonant to percussion, but rales were audible on examination of the bases. By percussion the pulmonary conus was prominent and the heart was enlarged to the right. The second pulmonic sound was accentuated and a systolic murmur was heard along the left sternal border. Generalized atrophy of the skeletal musculature and myotactile irritability confirmed the history of extensive loss of weight. Roentgenographic study of the chest showed soft infiltrations of both lung fields with increased bronchovascular markings. There was prominence of the right auricle and pulmonary conus (figure 1e). The red blood cell count was 5.5 million per cu. mm., and the hemoglobin concentration was 18.5 Gm. per 100 cc. (Sahli). The urine showed a one plus reaction for albumin, and the blood urea nitrogen was 27 mg. per 100 cc. Bromsulfthalein test showed 60 per cent retention at the end of forty-five minutes, after a dosage of 5 mg. per Kg. of body weight. Electrophoretic analysis of plasma protein was unremarkable save for slightly low albumin of 3.46 Gm. per 100 cc. and an increased concentration of alpha-2 globulin. A vitamin A absorption test was abnormal. The maximum breathing capacity was 51.5 liters per square meter, or 73.5 per cent of the predicted value (table 2). The lung volumes showed a very great reduction in vital capacity to 39 per cent, and total capacity to 56 per cent of the predicted values (table 2, and figure 2). The electrocardiogram was abnormal because of right axis deviation, and peaked P waves in lead II in addition to sinus tachycardia. The venous pressure was increased to 135 cm. and fell paradoxically to 11.5 cm. with pressure over the right upper quadrant of the abdomen. The arm-to-lung circulation time was prolonged to 11 seconds; arm-to-mouth time was 16 seconds. Venous catheterization of the right heart showed increased right auricular pressure (13 mm.) and right ventricular pulse pressure was as high as 78 mm. of mercury. The cardiac output by direct Fick principle was abnormally low or 3.1 liters per minute. Arterial oxygen saturation was depressed to 82 per cent with a pO_2 of 50 mm. Breathing pure oxygen increased these values to 95.6 per cent and 114 mm., respectively, but probably did not improve tissue utilization since the A-V oxygen difference fell from 9.4 to 7.78 vol. per cent. Exercise tolerance studies showed a marked impairment of pulmonary efficiency in relation to work, and this was aggravated by any increment over the resting value since exercise caused an increase in ventilatory equivalents (figures 3, 5, table 4). This unusual response was due to the extraordinary degree of hyperventilation induced by effort, and may also indicate further increments in pulmonary hypertension. The patient was unable to perform even 5 per cent grade walking except at the slow speed of 1.7 mph. Both ventilation indices and oxygen debts were abnormally high for either level or grade walking (table 4).

This fifth patient exhibited the late sequelae of chronic pulmonary granulomatosis due to beryllium, namely: contracted lungs with relative emphysema, cor pulmonale with incipient congestive failure, malnutrition, liver disease, and early renal disease in addition to severe anoxemia. Despite the latter, there was no marked polycythemia (figure 4). The ease with which such a patient may suffer serious complications is revealed by the following observations:

During the course of nitrogen balance studies, the patient was given 25 mg. of testosterone propionate intramuscularly daily for twelve days to ascertain its anabolic effects. The patient gradually became edematous, suffered more intense dyspnea, coughing and orthopnea even after the drug had been discontinued for a week. The laboratory evidence of congestive heart failure is presented in table 5. During the course of a second venous heart catheterization, the initial effects of 1.0 mg. of digoxin intravenously were recorded (figure 6). There was a transient lowering of right auricular pressure followed by an increase in right ventricular pulse pressure, and improvement in pressure pattern. Within forty minutes the right heart output improved sufficiently to overload the pulmonary circulation and pulmonary edema ensued. Fortunately the patient was promptly relieved by a phlebotomy

TABLE 5

Effects of congestive heart failure in patient J. J.

	APRIL 1948	MAY 1948	AFTER DIGOXIN THERAPY
Basal Weight, Kg	52.1	58.1	54.2
Lung volumes in liters			
Vital capacity	1.51	1.31	—
Residual air	1.23	1.50	—
Total capacity	2.74	2.81	—
Per cent residual air	45 per cent	53 per cent	—
Venous pressure			
cm. water	13.5	20.5	11
Circulation times, seconds			
Arm-to-lung	11	21	10
Arm-to-mouth	16	25	18
Resting metabolic rate	+19 per cent	+32 per cent	+43 per cent
Exercise tolerance			
1.7 mph, 0 per cent grade			
Endurance, minutes	10	8	10
Ventilation index	25	32	33
Oxygen debt	12 per cent	22 per cent	17 per cent
Average exercise			
Ventilatory equivalents	3.9	4.4	3.9
Heart rate	146	128	122
Blood pressure	141/79	118/83	143/77
Pulse pressure	62	35	66
Symptoms	Dyspnea	Severe dyspnea, exhaustion	Moderate dyspnea
Ballistocardiogram			
Pattern	Prominent H	Irregular	—
Amplitude	fair	low	—
Arterial Blood			
pCO_2, mm	41	34	—
pO_2, mm	50	46	—
O_2 saturation	82 per cent	82 per cent	—
O_2 capacity, vol. per cent	17.47	16.89	—
A-V O_2 difference, vol. per cent	9.40	5.84	—
Cardiac output, L/Min (breathing oxygen)	4.12	4.60	—
Maximal pulse pressure			
Right auricle	13	25	—
Right ventricle	78	68	—

of 350 ml. of blood, was then able to breathe easily while lying in the supine position, and rejected further supplemental oxygen therapy. The observed electrocardiographic K constant ($QT/\sqrt{\text{cycle length}}$) progressively shortened from 0.432 to 0.342 during the same period of time; inversion of T waves in lead II and occasional ventricular premature beats

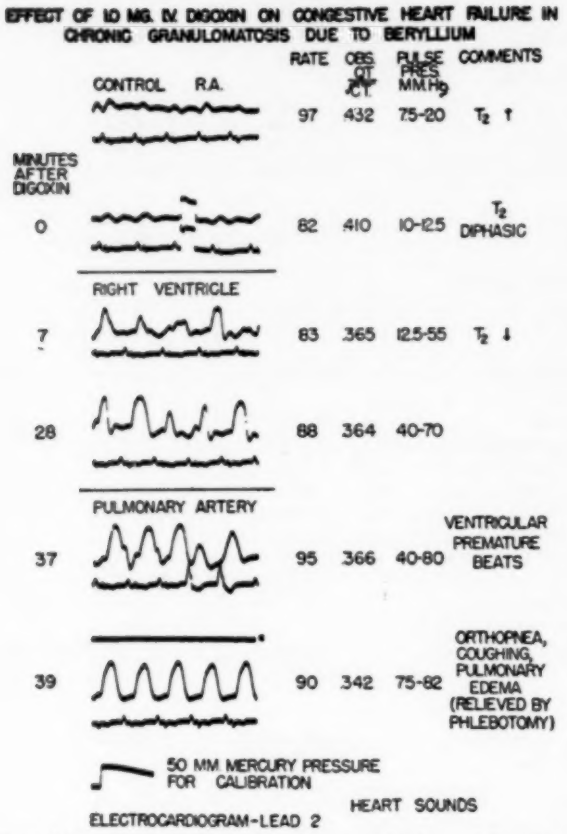


FIG. 6. Effect of digoxin on manifestations of congestive heart failure in patient J. J.; 1.0 mg. digoxin was administered intravenously during the second cardiac catheterrization. (Note irregular right ventricular pressure pulse patterns which were present prior to the administration of the digoxin, as well as typical electrocardiographic changes related to the digoxin).

further indicated the effects of digoxin on the myocardium. The cardiac output increased from 4.6 to 7.8 liters per minute. Diuresis was instituted promptly by the digoxin, and during the first twenty-four hours the basal weight declined by 1.75 Kg. The venous pressure was reduced to 11.0 cm. of water, and the arm-to-lung circulation time shortened to 10 seconds. Patient was thereafter continued on maintenance doses of digitoxin orally. An exercise tolerance test was repeated, and the patient was able to walk on the level the full 10 minute period at 1.7 mph and experienced much less dyspnea. The exercise systemic

pulse pressure was increased to the precongestive-failure level, and the ventilatory equivalent for oxygen was restored to its former value.

These data clearly show how long-standing beryllium granulomatosis can be complicated by cor pulmonale and congestive heart failure. The response to rapid digitalization was quite unusual because of the resistance to pulmonary blood flow which precipitated pulmonary edema requiring a phlebotomy to obtain relief. Such a patient needed continuous digitalis therapy to combat the heart failure.

The extraordinary disability that can result from overwhelming anoxemia is revealed by the sixth patient in this series:

W. R., a 35 year old white married male, was employed in the fluorescent light industry as an engineer. In March, 1945, he first noted exertional dyspnea which progressed in severity. Later he felt irritable, unstable, had anorexia, and during the summer lost 40 pounds in weight. Abnormal markings were found in the chest roentgenogram. Because of orthopnea and paroxysmal coughing, he was given penicillin and streptomycin by inhalation therapy in July, 1947 without striking benefit. Clubbing of the fingers had been present since 1946, and occasionally he noted slight dependent edema. He had had frequent passages of kidney stones for two years. For the six months prior to admission it had been necessary for him to use oxygen by an oral tube continuously to minimize dyspnea. On examination in May, 1948, he appeared to be a severely, chronically ill, white male with extensive wasting from malnutrition. He exhibited respiratory distress even at rest, and this was accentuated by any cause for anxiety. He showed variable tachypnea, dyspnea, orthopnea, as well as very marked clubbing and appreciable cyanosis even while breathing oxygen by a tube in his mouth. With persuasion the maximum possible time that he could tolerate breathing room air was about 20 minutes early in the course of observation, but later this time was less than a minute (figure 7). The chest was somewhat enlarged and the accessory muscles of respiration were used prominently. Breath sounds were of emphysematous character with wheezing and basilar rales bilaterally. The heart was not enlarged, the rhythm was regular, and the second pulmonic sound was accentuated. A faint systolic murmur as well as an early decrescendo diastolic murmur was audible along the left sternal border. Roentgenographic examination of the chest showed diffuse granular and confluent shadows bilaterally but more marked on the right side (figure 1f). The hematocrit was reduced to 37.5 per cent and the urine contained considerable albumin (3 plus reaction), and both red blood cells and white blood cells. The patient frequently passed kidney stones, and spectroscopic examination revealed beryllium within the stones.[7] The blood carbon dioxide combining power was increased to 77 vol. per cent and the chloride was depressed to 88 m.eq. per liter. Two-plus cephalin flocculation and thymol turbidity reactions indicated abnormal hepatic function. Bromsulpthalein retention was 40 per cent (repeated, 20 per cent) at the end of forty-five minutes. The observed blood volume was 5,600 ml., equal to the value predicted from height, but the plasma albumin concentration by electrophoresis was diminished to 2.7 Gm. per 100 cc., and all globulin concentrations except alpha-1 globulin were abnormal. The maximum breathing capacity was greatly depressed to 10.2 liters per square meter, or 15 per cent of the predicted value (table 2). The lung volumes showed marked reduction in total and vital capacities to 40 per cent and 53 per cent of values predicted (figure 2, table 2). The alveolar pressure-velocity relationships showed that less than the average pressure was needed to obtain moderate flow of air in the tracheobronchial tree (table 2). The electrocardiogram showed right heart strain with right axis deviation and inversion of T waves in leads II and III. Despite conspicuous distension of the peripheral veins, the venous pressure was repeatedly found to be between minus 0.5 and 2.0 cm. of water. The circulation times were normal. Venous catheterization of the right heart revealed increased pressure in auricle, ventricle, and

pulmonary artery (table 3). The cardiac output by the direct Fick principle with the patient breathing oxygen was increased to 5.92 liters per minute. Arterial oxygen saturation was 89 per cent with a pO_2 of 95 mm. The pCO_2 was increased to 56 mm. Breathing room air lowered the pO_2 to 46.2 mm. within a minute. The importance of oxygen therapy to this patient was further revealed by the rapid drop in arterial oxygen saturation based upon oximeter readings at 5 second intervals (figure 7). No exercise studies could be made on this patient.

This last patient, W. R., exhibited contracted lungs with relative emphysema, marked arterial anoxemia and cor pulmonale. In addition he suffered from mal-

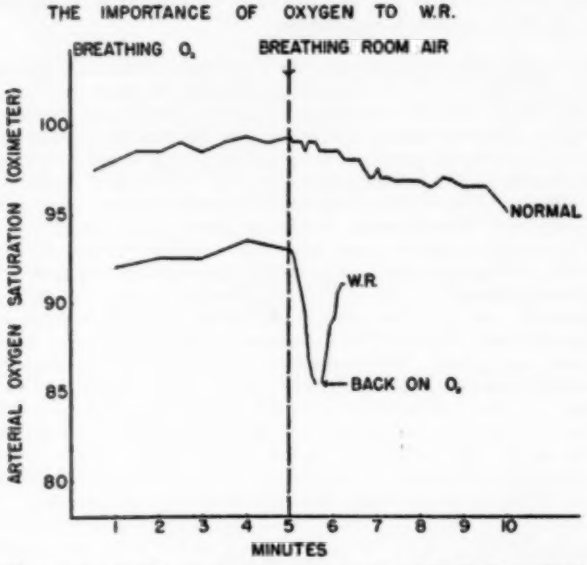


FIG. 7. Changes in arterial oxygen saturation on patient W. R. determined by oximeter readings while breathing oxygen and room air. (Note prompt decline in saturation in 35 seconds at which point the patient demanded restoration of oxygen.)

nutrition, wasting, hepatic, and renal disease. He required continuous oxygen therapy, and had moderate anemia rather than any compensatory polycythemia. The hematuria from renal lithiasis was partly responsible for the anemia. Yet there was no evidence of congestive heart failure, and in fact the venous pressure was subnormal. With deep inspiration the right ventricular diastolic pressure was observed to fall as low as minus 20 mm. of mercury. Because of fear of inducing a fatal pneumothorax, no attempt was made to measure the intrapleural pressure directly which must have been extraordinarily low during inspiration. In order to ascertain if some of the findings might be affected by cytochrome C therapy, the following observations were made (table 6):

The initial effects of cytochrome C observed were a 32 per cent reduction in oxygen consumption but no significant change in either arterial oxygen saturation or tension. The

TABLE 6

*Effect of cytochrome C on patient W. R.**

	BEFORE	AFTER
Breath-holding time, seconds	5	20
Minute ventilation, liters/M²/minute	5.98	6.44
Ventilatory equivalent for oxygen, liters/minute	2.36	3.73
Oxygen consumption, ml/M²/minute	253	171
Arterial Blood		
Carbon dioxide tension, mm.	75	—
Oxygen tension, mm.	67	76
Carbon dioxide, vols. per cent	79.6	76.9
Oxygen content, vols. per cent	12.6	12.6
Oxygen capacity, vols. per cent	14.5	14.7
Oxygen saturation, per cent	87	86
Mixed Venous Blood		
Carbon dioxide tension, mm.	84	85
Oxygen tension, mm.	35	38
Carbon dioxide, vols. per cent	80.5	78.8
Oxygen content, vols. per cent	8.65	8.85
Oxygen saturation, per cent	59	60
A-V carbon dioxide difference, vols. per cent	0.88	1.86
A-V oxygen difference, vols. per cent	3.93	3.75
Cardiac output liters/minute	6.42	4.56
Heart rate	98	118
Stroke volume, average ml.	65	39
Electrocardiogram	$T_{1,2}$—flat	T_1—elevated, T_2—depressed
K constant $\frac{QT}{\sqrt{CL}}$	0.425	0.403
Pneumocardiogram, Chest		
Beginning of right ventricular ejection, sec.	0.07	0.05
Beginning of left ventricular ejection	0.13	0.12
Mean right auricular pressure, mm. Hg.**	+2.8	−0.74
Mean right ventricular pressure, mm. Hg	23	24.6
Right ventricular pulse pressure, mm. Hg	66	84

* 80 mg. were administered through a catheter inserted into the right heart. Observations were made immediately before and one-half hour after catheterization while patient was breathing oxygen.

** Zero reference point 5 cm. below level of sternum.

cardiac output declined 29 per cent, with a rise in heart rate and a fall in stroke volume. The sytemic blood pressure rose from 102/72 to 126/100 mm. of mercury. The right ventricular pulse pressure rose from 66 to 84 mm. of mercury as the systemic pulse pressure fell

from 30 to 26 mm. of mercury. The electrocardiogram showed changes in T waves in the limb leads, and a reduction in the K constant. The beginning of ventricular systolic ejection, as indicated by simultaneous electrocardiogram and chest pneumocardiogram, shortened from 0.07 to 0.05 sec. after the beginning of the QRS complex for the right ventricle, and from 0.13 to 0.12 sec. for the left ventricle. The A-V oxygen difference diminished slightly from 3.93 to 3.75 vol. per cent. Maintenance doses of 50 to 75 mg. of cytochrome C were given intravenously daily for five days and, except for doubtful reduction in oxygen consumption, no effects were observed. Symptomatically the patient was unable to describe any specific change.

Based on inconclusive observations in a single patient it is impossible to assess any specific benefit to cytochrome C therapy in this group of patients.

Determination of Alveolar-arterial Oxygen Gradient

Inasmuch as the impaired diffusion of oxygen from the alveolar spaces to the pulmonary capillaries is held to be the keystone to the pathological physiology of beryllium granulomatosis (8), the results obtained in 5 of these patients are graphically portrayed in relation to ventilation, hemoglobin concentration and arterial oxygen saturation (figure 4). In comparison with normal average ventilation volumes at rest, these patients exhibited variable degrees of compensatory hyperventilation ranging from 40 to 240 per cent. The latter value is that for patient W. R., but it is important to note that at the time of this observation he was unable to tolerate breathing room air for more than five minutes. The resting ventilation was still more than 50 per cent above normal even while he was breathing supplemental oxygen. The alveolar pO_2 values recorded in figure 4 are those calculated from Riley's formula:

$$\text{Estimated alv. } pO_2 = (\text{tracheal } pO_2)\,\frac{\text{Exp. } N_2 \text{ per cent}}{\text{Insp. } N_2 \text{ per cent}} - \frac{\text{Art. } pCO_2}{\text{exp. R. Q.}}$$

In every instance but that of patient M. H. C., the observed mid-capacity pO_2 (by means of continuous sampling during breathing with a mask) was appreciably higher than the calculated alveolar value (table 7). The calculated alveolar-arterial pO_2 gradient (calc. alv. pO_2 minus observed arterial pO_2) was significantly increased in all patients, ranging from 24 mm. in patient M. E. T. to 50 mm. in patient W. R. It should be noted that the lowest gradient was found in patient M. E. T. who had the highest level of hemoglobin, 14.8 Gm. per 100 cc. None of these 5 patients exhibited a compensatory polycythemia, in fact 2 or 3 showed minor degrees of anemia. This, of course, contributed to the hypoxemia as well as obscuring frank evidence of visible cyanosis. Thus in these 5 patients, overbreathing was the sole compensatory mechanism for hypoxemia due to increasing the alveolar-arterial oxygen gradient. Dividing the observed oxygen consumption by the alveolar-arterial oxygen gradient, based upon the calculated value for alveolar pO_2, yielded diffusion constants for oxygen that were far below normal values (table 7) (22).

DISCUSSION

The causes of dyspnea found in these patients with pulmonary granulomatosis are essentially similar to those observed by Wright and his associates on a simi-

lar group of patients. Overbreathing, present at rest, and accentuated by exertion, is the result of an increased alveolar-arterial oxygen gradient, hypoxemia, and contracted breathing capacity and total lung volume (particularly the complemental air partition). The latter change may increase the proportion of residual air, but the observed volume was not greatly increased in any of these patients. Thus the pulmonary insufficiency more nearly resembles that seen in patients with pulmonary fibrosis than in those in whom emphysema is the primary defect. Pathological examination of fatal cases has shown the presence of diffuse emphysematous alterations in lung structure intermingled with granulomatous infiltrations. Functional studies of these patients do not, of themselves, offer data that serve to differentiate granulomatosis from other pulmonary diseases of comparable severity. Such studies are of value in delineating the differences between patients of the same group and, when analyzed according to pulmonary efficiency in relation to work performed (figure 3), the sequence of

TABLE 7

*Resting ventilation, alveolar pO_2, alveolar-arterial oxygen gradient, and diffusion constant for oxygen**

PATIENT	RESTING VENTILATION	OXYGEN CONSUMPTION	CALCULATED ALVEOLAR pO_2	OBSERVED ARTERIAL		ALVEOLAR-ARTERIAL OXYGEN GRADIENT	DIFFUSION CONSTANT FOR O_2	VENTILATORY EQUIVALENT FOR O_2
				pO_2	pCO_2			
	L/M²/min.	*ml/M²/m*	*mm.*	*mm.*	*mm.*	*mm.*		
M. C.	4.72	140	111	74	47	37	2.94	3.37
M. E. T.	4.21	162	96	72	36	24	3.60	2.60
M. H. C.	6.80	168	120	78	25	42	4.20	4.03
J. J.	4.24	141	98	49	41	49	2.04	3.03
W. R.	10.21	161	96	46	48**	50	1.97	6.38

* All measurements made while breathing room air.

** pO_2 96 and pCO_2 66 mm. while breathing oxygen.

complications is more readily understood. The complications of pulmonary granulomatosis evolve as compensating mechanisms for hypoxemia and increased pulmonary resistance. The earliest response to hypoxemia is hyperventilation, whereas usually polycythemia is a response to long-standing hypoxemia. Although occasional secondary polycythemia (8) has been observed in patients with granulomatosis, only one (patient A. B.) of those studied here exhibited this phenomenon. The infrequency of this response is provocative and in only patient W. R. could it have been obscured by chronic blood loss (from renal lithiasis). It is possible that dietary deficiencies, inadequate absorption of constituents of hemoglobin, or impaired hemoglobin metabolism related to the generalized beryllium toxicity, may have been responsible. Of particular interest in this regard are the observations of other investigators who have noted the absence of polycythemia in patients with severe chronic anoxemia. Studies on 20 patients with pulmonary tuberculosis treated by thoracoplasty failed to show any evidence of polycythemia despite arterial anoxemia (23). Investigations of the

influence of anoxemia on the hemopoietic activity in healthy and diseased males at high altitudes have shown limitations to the hematologic response to the anoxic stimulus. Extreme anoxia results in a decrease in the polycythemic response which may be attributed to an interference with the formation of hemoglobin (24). Cor pulmonale develops after the onset of pulmonary hypertension. The earliest evidence of this is increased right ventricular pressure with exertion; the late manifestations are right heart strain and congestive heart failure. Data obtained on patient J. J. indicate that retention of water and electrolytes are important contributory factors to the appearance of failure. In addition, the too rapid treatment of congestive failure with intravenous digoxin can be hazardous when the pulmonary vascular bed is unable to accomodate an increased right heart output. Under these circumstances pulmonary edema was acutely precipitated, and just as quickly relieved by phlebotomy. Excessive exertion, such as grade walking, can cause acute transient myocardial failure as manifested by more severe dyspnea and markedly increased oxygen debt in patient M. C. Digitalis therapy appears to be of value only in the presence of congestion, for no greater exercise tolerance was exhibited by its use in patients without congestion. Orthopnea, however, is an expression of myocardial failure in either instance.

Many of the patients with pulmonary granulomatosis are distressed by daily fluctuations in intensity of dyspnea. In part these variations can be related to psychological adjustments to an occupational disease and the effects on their daily activities. As expected, anything that can induce anxiety in these patients aggravates their dyspnea. Finally more profound alterations in water and electrolyte metabolism, especially in relation to the menstrual cycle, may alter awareness of dyspnea. An example is the increased blood volume during the premenstrual phase associated with lessened vital and total pulmonary capacity and greater exertional dyspnea, presumably because of pooling of more blood within the lungs in patient M. E. T. In the same patient the ingestion of ammonium chloride during the menses resulted in weight loss, striking reduction in residual air, and decrease in arterial pCO_2 together with marked accentuation of dizziness. Despite these symptomatic variations, the ventilatory equivalent for oxygen, which expresses pulmonary efficiency, was the least variable datum in a series of multiple exercise tolerance tests performed on 2 patients, M. E. T. and M. H. C.

The secondary effects are those of malnutrition, liver, and renal disease. Appropriate metabolic studies will be the subject of a separate report by Dr. Christine Waterhouse. From preliminary observations it is not yet clear whether hypoxemia alone, or in combination with beryllium poisoning of enzyme systems, can account for these effects. Except for the necessity of maintaining patient W. R. against overwhelming anoxemia, the observed data appear to afford no evidence that any specific benefits were obtained from either oxygen or cytochrome therapy in these patients.

SUMMARY

The resting and exercise measurements of pulmonary and cardiovascular functions in 6 patients with chronic pulmonary granulomatosis are presented in rela-

tion to the evolution of the functional impairment and its complications. The results of these studies are essentially in accord with those observed by other investigators of a similar group of patients. The main deviation from normal is the hyperventilation, especially during work, compensating for hypoxemia. Diminished arterial oxygen saturation is a function of abnormally increased alveolar-arterial gradient for oxygen and contracted lung volume, particularly complementary air. One of these patients exhibited polycythemia as a compensatory mechanism. Various stages of cor pulmonale, including congestive heart failure due to increased pulmonary resistance, were observed. An unusual response to intravenous digoxin and the lack of benefit from cytochrome C therapy are recorded. Variations in response to exercise and similarities to functional impairment of other chronic pulmonary disease are discussed.

Addendum

Two more patients have been studied since the preparation of this report. Each one has presented findings similar to the others, but neither has been as far advanced as either J. J. or W. R.

SUMARIO

Observáciones Relativas a las Causas de la Dispnea en la Granulomatosis Pulmonar Crónica de los Trabajadores en Berilio

Las mediciones de las funciones pulmonar y cardiovascular en descanso y en ejercicio en 6 enfermos con granulomatosis pulmonar crónica se presentan en relacion con la evolución de la disfunción y sus complicaciones. El resultado de estos estudios concuerda esencialmente con lo observado por otros investigadores en un grupo semejante. La principal desviación de lo normal consiste en la hiperventilación, sobre todo durante el trabajo, que compensa la hipoxemia. La hiposaturación arterial para el oxígeno es efecto de la anormal hipergradiente alveoloarterial para el oxígeno y del disminuido volumen pulmonar en particular para el aire complementario. Uno de los enfermos manifestó policitemia como mecanismo compensador. Observáronse varias etapas del "cor pulmonale," incluso insuficiencia cardíaca congestiva debida a hiperresistencia pulmonar. Menciónanse una inusitada respuesta a la digoxina por vía venosa y la ineficacia de la citocromo-C-terapia. Discútense las varias reacciones al ejercicio y las semejanzas a la disfunción ocasionada por otras neumopatías crónicas.

REFERENCES

(1) Van Ordstrand, H. S., Hughes, R., DeNardi, J. M., and Carmody, M. G.: Beryllium poisoning, J. A. M. A., 1945, *129*, 1084.

(2) Hardy, H. S., and Tabershaw, I. R.: Delayed chemical pneumonitis occurring in workers exposed to beryllium compounds, J. Indust. Hyg. & Toxicol., 1946, *28*, 197.

(3) Higgins, H. S.: Pulmonary sarcoidosis, Connecticut M. J., 1947, *11*, 330.

(4) Pyre, J., and Oatway, W. H., Jr.: Beryllium granulomatosis, Arizona Med., 1947, *4*, 21.

(5) DeNardi, J.: Four neighborhood cases of chronic pneumonitis and two cases of chronic pneumonitis, probably of the delayed type (unpublished data, December, 1947).

(6) Editorial: Industrial beryllium poisoning, J. A. M. A., 1947, *135*, 840.

(7) WILSON, STANLEY: Personal communication (1948).
(8) WRIGHT, G. W., FILLEY, G. F., PROEMMEL, D. R., PRINCI, F., PLACE, T., AND GRINNELL, R.: Observations concerning the pathological physiology underlying exertional dyspnea in the disease "Granulomatosis occurring in beryllium workers", Symposium on Military Physiology, 1947, Digest Series No. 4, 135.
(9) MARTLAND, H. S., BRODKIN, H. A., AND MARTLAND, H. S., JR.: Occupational beryllium poisoning in New Jersey: With special reference to its incidence, diagnosis, and compensation, New Jersey Med. Soc. J., 1948, *45*, 5.
(10) BALDWIN, E. DEF., COURNAND, A., AND RICHARDS, D. W., JR.: Pulmonary insufficiency. I. Physiological classification, clinical methods of analysis, standard values in normal subjects, Med. 1948, *27*, 243.
(11) MENEELY, G. R., AND KALTRIEDER, N. L.: Use of helium for determination of pulmonary capacity, Proc. Soc. Exper. Biol. & Med., 1941, *46*, 266.
(12) RAHN, H., MOHNEY, J., OTIS, A. B., AND FENN, W. O.: A method for the continuous analysis of alveolar air, J. Aviation Med., 1946, *17*, 173.
(13) ZAVOD, W. A.: Bronchospirography. I. Description of the catheter and the technique of intubation, J. Thoracic Surg., 1940, *10*, 27.
(14) COURNAND, A., RILEY, R. C., BREED, E. B., BALDWIN, E. DEF., RICHARDS, D. W., LESTER, M. S., AND JONES, M.: Measurement of cardiac output in man using the technique of catheterization of the right auricle or ventricle, J. Clin. Investigation, 1945, *24*, 106.
(15) RILEY, R. L., PROEMMEL, D. D., AND FRANKE, R. A.: A direct method for determination of oxygen and carbon dioxide tensions in blood, J. Biol. Chem., 1945, *161*, 621.
(16) PETERS, J. P., AND VAN SLYKE, D. D.: Quantitative Clinical Chemistry. II. Methods, Baltimore, 1932.
(17) ROUGHTON, E. J. W., AND SCHOLANDER, P. F.: Micro-gasometric estimation of blood gases, J. Biol. Chem., 1943, *148*, 3.
(18) BROWN, H. R., JR.: Personal communication (1948).
(19) BLAIR, H. A., WEDD, A. M., AND HARDWICKE, H. M.: The normal pneumocardiogram, Am. J. Physiol., 1942, *136*, 523.
(20) LAMBERT, E. H., AND WOOD, E. H.: The use of a resistance wire, strain gauge manometer to measure inter-arterial pressure, Proc. Soc. Exper. Biol. Med., 1947, *64*, 186.
(21) BRUCE, R. A., LOVEJOY, F. W., JR., BROTHERS, G. B., AND VELASQUEZ, T.: Unpublished methods and normal standards (1948).
(22) KROGH, M.: The diffusion of gases through the lungs of man, J. Physiol., 1915, *49*, 271.
(23) KALTREIDER, N. L., FRAY, M. D., AND PHILLIPS, E. W.: The effect of thoracoplasty on the pathologic physiology of respiration, J. Thoracic Surg., 1938, *7*, 262.
(24) HURTADO, A., MERINO, C., AND DALZADO, E.: Influence of anoxemia on the hemopoietic activity, Arch. Int. Med. 1945, *75*, 284.

THE ROLE OF PULMONARY CAVITATION IN THE DEVELOPMENT OF BACTERIAL RESISTANCE TO STREPTOMYCIN[1, 2]

W. L. HOWARD, F. MARESH, E. E. MUELLER, S. A. YANNITELLI AND C. E. WOODRUFF

(Received for publication December 22, 1948)

INTRODUCTION

The use of antibiotics in combating infection is frequently limited by the capacity of microorganisms to develop resistant strains against which the drugs are no longer effective. This is particularly true in the case of a chronic disease such as tuberculosis and the antibiotic streptomycin which, though an excellent bacteriostatic agent, has little bactericidal effect. In the earliest reported *in vitro* studies, as well as the first attempts to use streptomycin in the treatment of human tuberculosis (1, 2), the development of highly resistant mycobacterial strains was noted. Though much work has been done on this subject during the past two years, there is still no clear cut explanation of the factors involved in the development of resistant strains of *M. tuberculosis*. It is evident that adequate knowledge of these factors would greatly increase the field of usefulness of streptomycin.

That the duration of therapy with streptomycin is one factor in causing the development of resistant bacilli has been shown in experimental animals. Steenken (3), after treating tuberculous guinea pigs with streptomycin for 125 days, recovered only nonresistant tubercle bacilli from the animals. Feldman, Karlson, and Hinshaw (4) obtained similar results when infected guinea pigs were treated for 60 days, but found that, if treatment was continued for 206 days or longer, tubercle bacilli resistant to more than 2,000 γ of streptomycin per ml. of media could be cultured from the spleen of each animal. Also the data from various clinics suggest the important role played by duration of therapy. A report from the Veterans Administration Hospitals (5) indicates that after 60 to 90 days of treatment bacilli resistant to 10 γ or more of streptomycin have developed in 64 per cent of the patients; after 120 days of therapy more than 78 per cent of the patients had developed resistant strains. The work of Youmans and Karlson (6) and that of Bernstein, D'Esopo and Steenken (7) similarly indicate the dangers with regard to bacillary changes inherent in long courses of streptomycin.

While the importance of long continued streptomycin therapy in causing resistant bacteria to develop is recognized, evidently other factors are of greater importance in some cases. For example, Wolinsky, Reginster and Steenken (8), in reporting on 47 patients treated with streptomycin, obtained one culture

[1] From the William H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium), Northville, Michigan.

[2] This investigation was supported (in part) by a grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

resistant to 1,000 γ of streptomycin per ml. of media after only 29 days of therapy, while cultures from 11 of the patients remained sensitive to 1.0 γ of streptomycin after 12 weeks of therapy. Howlett and O'Connor (9) observed that in 26 patients with disseminated nodular lesions who received streptomycin therapy the only one who eventually produced tubercle bacilli resistant to 10 γ of streptomycin was a patient who also had a persistent cavity. In this particular patient the bacilli eventually became resistant to 1,000 γ of streptomycin.

Prior to the publication of the last mentioned article, the casual observation was made at the Maybury Sanatorium that there seems to be a close correlation between the presence of cavity and the development of resistant tubercle bacilli. To test this observation more carefully, an analysis has been made of the data obtained from 155 streptomycin-treated patients who have had posttreatment resistance studies performed on tubercle bacilli isolated from their sputum or gastric contents.

MATERIALS AND METHODS

The 155 patients included in the present study represent approximately one-half of the adult patients with pulmonary tuberculosis who were treated with streptomycin at the Wm. H. Maybury Sanatorium during the interval from November, 1946 to August, 1948. The only patients excluded from the study are those on whom no posttreatment determinations of bacterial sensitivity to streptomycin are available. For most of the sensitivity determinations we are indebted to Dr. Cora R. Owen[2] of the University of Michigan and to Dr. Guy P. Youmans[3] of Northwestern University.

During the twenty month period of the study, six significantly different streptomycin dosage schedules were employed; 2 Gm. per day given in divided doses of 0.4 Gm. five times daily for 90 days; 1.0 Gm. per day given in divided doses of 0.5 Gm. twice daily to a total of 100 Gm.; 1.0 Gm. daily given as a single dose for 42 days; 0.5 Gm. daily given as a single dose for 84 days; a dose varying from 0.4 to 0.7 Gm., depending upon the patient's weight and given twice daily for 84 days. In one small series of patients streptomycin was given during alternate weeks according to the schedule last mentioned.

Chest roentgenograms were taken at the beginning of therapy and thereafter at intervals of four weeks in most cases; in the case of patients whose therapy was terminated at the end of six weeks the roentgenogram was taken at that time.

In preparing the correlation tables only a single bacterial sensitivity determination has been used for each patient except in 11 instances. In these 11 cases, 2 sensitivity determinations, made on bacteria isolated from a single patient at intervals longer than four weeks, have been used. Thus 166 sensitivity determinations, made on bacteria isolated from 155 patients, are included in the study. In addition to these 166 determinations made during the course of streptomycin therapy, or after its completion, 7 pretreatment determinations were done. All 7 of these cultures failed to grow in media containing one γ of streptomycin per ml.

Data regarding the presence or absence of cavitation were obtained from the interpretation of the roentgenologist as recorded in each patient's chart. If the interpretation was equivocal on this point, the original roentgenogram was consulted. The pertinent roentgenogram was the one taken at the same time, or near the time, that tubercle bacilli were isolated from the patient's sputum for the sensitivity determination. Since most of the sensitivity tests were made at the termination of therapy, this means that in most cases the patient's roentgenogram taken at the *conclusion* of his course of streptomycin determined whether or not he should be classified as having a cavity.

RESULTS OF STUDY

The impossibility of classifying all cases as definitely showing or not showing cavitation soon became apparent. As mentioned above, the original roentgeno-

grams were consulted whenever the recorded reading of the roentgenologist was found equivocal. Even with the additional information obtained from subsequent roentgenograms, however, it was frequently impossible to say whether or not at a given time definite cavitation was present. Thus, instead of separating the cases into two groups, those with and those without cavities, it was found necessary to add a third group of cases in which the findings with regard to the presence or absence of cavities remained equivocal. In the first group, those showing definite cavitation at the time the sensitivity determination was made, there are 88 cases. Thirty-seven cases are found in the "equivocal" group, while 41 cases are included in the noncavity group. In 11 instances, patients who showed definite cavitation at the beginning of therapy had completely closed the cavity by the time the sensitivity determination was made. In 21 instances, patients with cavitation at the beginning of therapy were finally placed in the "equivocal" group. Changes in the reverse direction occurred only four times

TABLE 1

Data from cases showing cavitation at the end of streptomycin therapy

RESISTANCE TO STREPTOMYCIN	DURATION OF THERAPY IN DAYS			TOTALS		
	28 to 41	42 to 83	84 to 160	Number	Per cent	
	Number of cultures					
γ *per ml.*						
<1	0	4	0	4	4.5	
1–10	5	5	0	10	11.4	15.9
10–100	2	12	10	24	27.3	
100–1,000	2	7	13	22	25.0	
>1,000	3	17	8	28	31.8	84.1
<1->1,000	12	45	31	88	100.0	100.0

while patients were taking streptomycin and in four instances patients who had no cavity at the beginning of therapy had roentgenograms which were considered "equivocal" at the time the sensitivity determination was made.

With 6 exceptions, every patient who had definite cavitation at the beginning of therapy had sputum which was positive for acid-fast bacilli on direct smear. The 6 exceptions were "positive" only on culture of the sputum.

The data for the three groups of patients, classified according to duration of therapy and the level of streptomycin resistance of the mycobacteria isolated from the sputum, are shown in tables 1, 2, and 3.

Following established custom (5), it is assumed that tubercle bacilli able to grow in media containing 10 γ of streptomycin per ml. are *resistant* as far as effective clinical therapy is concerned. Using this definition of resistant microorganisms, it will be seen from table 1 that of the patients showing cavitation the great majority (84.1 per cent) produced resistant tubercle bacilli. Among the cultures obtained 28 to 41 days after the beginning of therapy, 7 of 12, or 58.3

per cent, were resistant. In the 42 to 83 day group, 36, or 80 per cent of resistant cultures, were found and in the 84 day group *all* of the cultures were resistant.

It will be noted that *no* cultures of tubercle bacilli *sensitive* to one γ or less of streptomycin were found in the 28 to 41 day group, while four cultures of this type were found among patients who had had therapy for 42 to 83 days. This obvious aberration can be explained on the basis of the composition of the various

TABLE 2

Data from cases in which the evidence of cavitation remained equivocal

RESISTANCE TO STREPTOMYCIN	DURATION OF THERAPY IN DAYS			TOTALS		
	28 to 41	42 to 83	84 to 160	Number	Per cent	
	Number of cultures					
γ *per ml.*						
<1	1	7	0	8	21.6	
1–10	2	20	3	25	67.6	89.2
10–100	0	1	0	1	2.7	
100–1,000	0	1	0	1	2.7	
>1,000	0	1	1	2	5.4	10.8
<1–>1,000	3	30	4	37	100.0	100.0

TABLE 3

Data from cases showing no cavitation at the end of therapy

RESISTANCE TO STREPTOMYCIN	DURATION OF THERAPY IN DAYS			TOTALS		
	28 to 41	42 to 83	84 to 160	Number	Per cent	
	Number of cultures					
γ *per ml.*						
<1	3	10	6	19	46.3	
1–10	2	13	5	20	48.8	95.1
10–100	0	1	1	2	4.9	
100–1,000	0	0	0	0	0	
>1,000	0	0	0	0	0	4.9
<1–>1,000	5	24	12	41	100.0	100.0

groups as regards dosage schedule. In the first group, the majority of the 12 patients had been on the 0.5 Gm. schedule, *i.e.*, they had received 0.5 Gm. of streptomycin daily in a single dose. The 42 to 83 day group included only one patient who had been on the 0.5 Gm. schedule and 34 who had received 1.0 Gm. daily in a single dose. The remaining 10 patients had received either 0.5 Gm. twice daily or 2.0 Gm. daily divided into five doses. In the light of these different dosage schedules, the complete absence of any cultures in the 28 to 41 day group sensitive to one γ or less of streptomycin suggests that the daily dose of 0.5 Gm.

of streptomycin *for patients having pulmonary cavities* merely stimulates the population of tubercle bacilli present in the cavities to an early development of resistance.

An observation similar to the above is mentioned by Steenken (10). After 46 to 90 days of therapy, he found that a greater proportion of patients receiving 0.5 Gm. of streptomycin per day had produced resistant tubercle bacilli than was the case for those patients receiving either 1.0 Gm. or 1.8 Gm. daily. Also pertinent to this question is the report of Wolinsky and Steenken (11) that tubercle bacilli begin growing *in vitro* with little or no delay in a culture medium

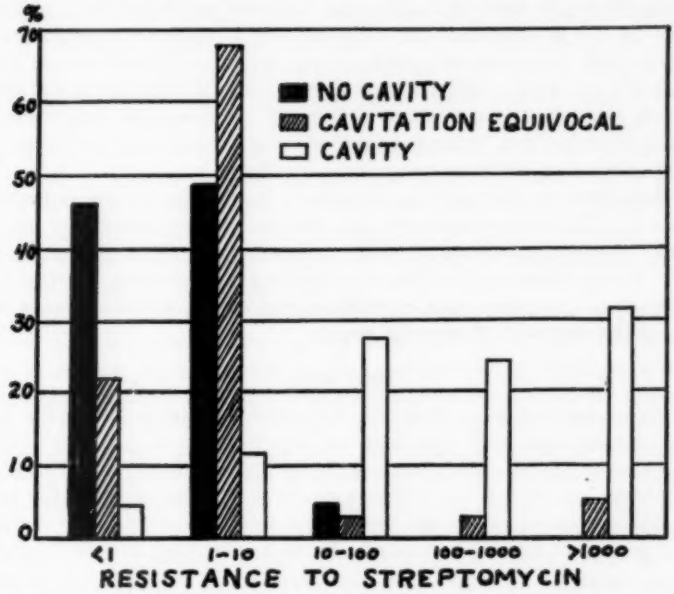


FIG. 1. Per cent of cultures showing resistance to streptomycin as related to persistent cavitation. (From sensitivity determinations on 166 cultures obtained from 155 patients treated with streptomycin.)

containing 0.1 γ of streptomycin per ml., while there is no growth for more than two weeks in the same medium containing 0.2 γ of streptomycin per ml.

To be noted in table 1 is the fact that in 3 instances tubercle bacilli resistant to more than 1,000 γ of streptomycin were found after only 28 to 41 days of therapy. All 3 of these patients had received the minimal dose of 0.5 Gm. of streptomycin daily, one of them for only 28 days, the other 2 for 34 days. All 3 had large cavities measuring from 5 to 10 cm. in diameter.

Table 2 shows that 10.8 per cent of the patients in whom the finding with regard to cavitation was equivocal developed resistant strains of tubercle bacilli. In 3 instances the original area of excavation became opaque to the roentgen

rays and was interpreted as a "filled" cavity. The bacilli from 2 of these patients were sensitive to 10 γ of streptomycin; bacilli from the third fell in the 10 to 100 γ per cc. resistance group.

In table 3 only 2 patients (4.9 per cent of the total) are found to have developed resistant strains. One of these 2 patients had tuberculous pericarditis as well as disseminated pulmonary disease; the other had received streptomycin for 115 days, except for one intermission of two weeks, when the resistant culture was obtained.

Of further interest in table 3 is the fact that 6 patients, after treatment with streptomycin for 84 days or longer, still harbored tubercle bacilli which were sensitive to 1 γ of streptomycin. Five of the 6 began their treatment with recently formed cavities which closed during the course of streptomycin therapy; the sixth did not show a cavity at any time. Most of these particular patients have been mentioned in a previous article (12). Subsequent follow-up shows that all 6 have become "sputum-negative"; their roentgenograms have shown progressive clearing and their prognosis is excellent.

The data from the final columns of tables 1, 2 and 3 are presented graphically in figure 1. This chart emphasizes the fact that in the presence of persistent cavitation the development of resistant strains of tubercle bacilli is a significant matter. In the absence of cavitation at the end of treatment, resistant strains rarely develop. In those cases in which cavitation is equivocal there is still no significant development of resistant strains.

DISCUSSION

The data presented above illustrate the marked variation observed in clinical cases of tuberculosis with regard to the development of resistant strains of tubercle bacilli. In one group of cases the bacilli remained sensitive to one γ of streptomycin even after 84 days of therapy; in another group resistance to more than 1,000 γ of streptomycin developed after only four to six weeks of therapy. What is the reason for this variation? Is the development of resistant tubercle bacilli, as suggested by Yegian and Vanderlinde (13), merely a consequence of the ". . . selection of normally occurring hereditary variants that are present in the original cell population"? If this were true, then the bacilli of a patient in the first group would remain sensitive because that patient's "original cell population" did not contain resistant cells, whereas a patient in the second group would be unlucky because amongst his original quota of bacilli there happened to be highly resistant cells. In the light of the data which have been presented, this explanation seems unlikely. There is too close a correlation between persistent cavitation and the development of resistant bacilli to be explainable on the basis of mere chance variation in the original cell population.

One obvious reason for the importance of the cavity in determining whether or not resistant bacilli will appear is the well known fact that tubercle bacilli grow much more readily and more rapidly in this location where they have been "exteriorized" as far as the ordinary defense mechanisms of the body are concerned (14). Rapid multiplication is generally accepted as important in the

development of resistant microorganisms (2, 15). Another important characteristic of tuberculous cavities, particularly the older, more chronic variety, is that their relatively avascular walls (16) militate against streptomycin reaching the interior of the cavity in effective concentration. In one experimental study (17) the streptomycin content of sputum from the cavities of patients under therapy with 1.8 to 2 Gm. of streptomycin daily was found to vary from a minimum of 1 γ per ml. to a maximum of 18 γ per ml.

Finally, within the tuberculous cavity the tubercle bacillus is frequently able to grow in a fluid or semifluid menstruum instead of on the surface of the cells, and this may be of considerable importance in bringing about the rapid evolution of highly resistant strains. Steenken has stated (18) that strains of tubercle bacilli resistant to streptomycin can be produced much more rapidly when the microorganisms are grown in the depths of a liquid medium such as that of Dubos (19) than when they are grown as a pellicle on the surface of a medium. Ballon and Guernon (20, 21) and Follis (22) have shown that sulfanilamide, incorporated in a solid medium, completely inhibits the growth of tubercle bacilli while sulfanilamide in the same concentration in a liquid medium has no inhibitory effect. These observations suggest that strains of tubercle bacilli resistant to sulfanilamide may evolve when the bacteria are grown in liquid but not on solid media containing the drug.

From the data of the present study it is evident that strains of tubercle bacilli highly resistant to streptomycin may be produced very rapidly in some cases while in other cases the increase in resistance on the part of the tubercle bacillus appears to be a gradual process. Demerec (23) has noted a similar behavior in the case of the staphylococcus. When this microorganism is submitted to the action of streptomycin for a single time, some strains are produced which are almost completely resistant while other strains are only slightly more resistant than the parent strain. Demerec explained this erratic sort of increase in resistance to streptomycin on the basis of mutations in genes varying greatly in their potency, a mutation in a highly potent gene being responsible for a high degree of resistance, a mutation in a less potent gene for a low degree of resistance. The hypothesis that similar changes may occur in the case of the tubercle bacillus offers an attractive explanation for the observed facts.

Just how rapidly may highly resistant strains of tubercle bacilli appear? Vennesland *et al.* (24) have demonstrated that tubercle bacilli 10,000 times as resistant to streptomycin as the original strain may originate from a stock laboratory strain of H-37Rv after its exposure to dilute streptomycin for 21 days in Dubos medium[3]. With regard to clinical studies, one patient in the present series discharged tubercle bacilli resistant to 1,000 γ of streptomycin after 28 days of therapy. Wolinsky, Reginster and Steenken (8) have reported that a strain similarly resistant appeared after 29 days of therapy, and McDermott (25) noted the appearance of a highly resistant strain after five weeks of therapy. The most specific example of the rapid emergence of resistant tubercle bacilli

[3] This interpretation is different from that of Vennesland *et al.*, who considered that naturally occurring streptomycin-resistant variants had been demonstrated.

has been mentioned by Karlson (26) who made sensitivity tests on tubercle bacilli isolated on the dates mentioned below from the sputum of a patient receiving streptomycin therapy[4].

Date of sputum culture	*γ of streptomycin per ml. of culture medium necessary to inhibit growth*
3-22-46	0.31
3-28-46	0.31
4- 1-46	0.31
4-10-46	0.31
4-17-46	0.31
4-26-46	0.31
5- 8-46	> 1,000

Thus, after remaining sensitive to 0.31 γ of streptomycin for approximately six weeks, the tubercle bacilli isolated from this particular patient only 12 days later were resistant to more than 1,000 γ of streptomycin.

It is evident that the cumulative effect of numerous small step mutations (such as were observed by Demerec), should they occur in the tubercle bacillus, would eventually produce highly resistant bacilli. Against this explanation in the present case is the fact that the growth of the tubercle bacillus is necessarily slow, too slow to allow any large number of generations to occur during the 12 day interval observed by Karlson.

In vitro experimental studies indicate that different growth mechanisms may operate for the tubercle bacillus, depending on whether the microorganism is grown in liquid or on solid media. Kahn (27) observed that tubercle bacilli growing in a liquid medium pass through a developmental cycle which requires 17 days for its completion. Tubercle bacilli growing on solid media do not pass through any such cycle but appear to proliferate by direct fission (28). As long as mycobacteria are growing by direct fission, the mycelium which is formed might be compared to the continuous growth of one plant, and no great variation in the individual cells would be expected. However, when the bacillus passes through a complex cycle of development, there is greater opportunity for the mutation of highly potent genes which, in the present case, could bring about abruptly the appearance of a strain of bacilli highly resistant to streptomycin. Of interest in this regard is the observation of Wolinsky and Steenken (11) that tubercle bacilli exposed in a liquid medium to 0.2 γ of streptomycin per ml. show no evidence of growth for the first *17 days*; after this period growth is rapid. The appearance of the 17 day interval in the above work and in the cycle observed by Kahn may be more than a mere coincidence. Also, the 21 day interval found necessary by Vennesland *et al.* (24) for the production of tubercle bacilli highly resistant to streptomycin is long enough to include the 17 day growth cycle.

Returning to experimental tuberculosis in guinea pigs, Feldman, Karlson, and

[4] In following the development of bacterial resistance in streptomycin-treated patients, Steenken (18) has noted that the cultures isolated from a given patient may change from sensitive to 0.5 γ of streptomycin per ml. to resistant to 1,000 γ per ml. in a period of only seven days.

Hinshaw (4), as already mentioned, found that treatment of the animals with streptomycin for a long period of time (206 days) was necessary before highly resistant strains of tubercle bacilli appeared. In this respect tubercle bacilli in the guinea pig react much as they do in the human patient who has no pulmonary cavity. The reason may be the same in both instances, for cavitation is almost never seen in ordinary inoculation tuberculosis of guinea pigs.

The practical implications of the close correlation observed between persistent cavitation and the development of resistant strains of tubercle bacilli are obvious. If there is no cavitation in cases of minimal tuberculosis, the usual course of streptomycin might be given to such patients with little or no fear that resistant bacilli will develop. If cavitation is present and closure of the cavity occurs during the course of therapy, again there is very little danger of the development of resistant strains of tubercle bacilli. These facts should influence the therapeutic strategy of the physician with regard to each patient; his every effort should be directed toward obtaining cavity closure before completion of the streptomycin therapy. If the cavitation is very extensive or of such a character that it may not close during the course of streptomycin, the drug should supplement collapse therapy rather than precede it.

One other implication has to do with the question of streptomycin-resistant tubercle bacilli in patients who have become "sputum negative." These are the patients who have shown such a good response to streptomycin that it is impossible to recover tubercle bacilli from their sputum at the end of the course of therapy. Fears have frequently been expressed, however, that these patients might be harboring highly resistant tubercle bacilli in some obscure focus in the body. In view of the almost complete absence of resistant bacilli in patients without cavitation, these fears seem unfounded.

Data regarding the development of streptomycin-resistant tubercle bacilli in relation to the various dosage schedules employed in the present series of cases are too fragmentary to permit any observations other than that the dose of 0.5 Gm. of streptomycin per day *in cases showing cavitation* appears to be too small to prevent the rapid emergence of highly resistant strains of bacilli. Looking upon the cavity wall as a semipermeable membrane (relatively impermeable in the case of thick-walled cavities), it is evident that a high streptomycin blood level, if maintained for only a few hours, might be more effective in producing a satisfactory level of streptomycin within the cavity than a lower blood level maintained for a longer period of time. Once a fair concentration of streptomycin has been obtained in the contents of the cavity, this level might be expected to remain fairly constant (even after the blood level has fallen) until the cavity is emptied.

Apparently a single 1.0 Gm. dose of streptomycin produces a satisfactorily high blood level (29). Thus 1.0 Gm. of streptomycin given in a single dose on alternate days might be considerably more effective than 0.5 Gm. of the drug given daily. Such a method of "concentrated therapy" at relatively long intervals might make it possible to avoid the cumulative toxicity of long continued daily dosage with streptomycin while maintaining an effective attack against the tubercle bacillus.

SUMMARY AND CONCLUSIONS

One hundred fifty-five cases of pulmonary tuberculosis treated with streptomycin have been analyzed with regard to the emergence of resistant strains of tubercle bacilli in relation to the presence or absence of cavities. From the analysis the following conclusions seem justified:

(*1*) In the presence of persistent cavitation the emergence of resistant strains of tubercle bacilli is a significant matter.

(*2*) In the absence of cavitation at the end of the course of therapy, resistant strains rarely appear.

(*3*) In those cases in which cavitation is equivocal the emergence of resistant strains of tubercle bacilli occurs with appreciably less regularity than in the presence of definite cavitation.

SUMARIO Y CONCLUSIONES

El Papel de las Cavernas Pulmonares en la Producción de Resistencia Bacteriana a la Estreptomicina

Cinento cincuenta y cinco casos de tuberculosis pulmonar tratados con estreptomicina son analizados con respecto a la emergencia de cepas resistentes de bacilos tuberculosos en relacion con la presencia o ausencia de cavernas. El análisis parece justificar las siguientes conclusiones:

(1) En presencia de cavernas persistentes la emergencia de cepas resistentes de bacilos tuberculosos es significativa.

(2) En ausencia de cavernas rara vez aparecen cepas resistentes al terminar la terapéutica.

(3) En los casos en los que es dudosa la presencia de cavernas, la emergencia de cepas tuberculosas resistentes ocurre con regularidad apreciablemente menor que en presencia de cavernas bien definidas.

REFERENCES

(1) Youmans, G. P., Williston, E. H., Feldman, W. H., and Hinshaw, H. C.: Increase in resistance of tubercle bacilli to streptomycin: A preliminary report, Proc. Staff Meet., Mayo Clinic, 1946, *21*, 126.

(2) Middlebrook, G., and Yegian, D.: Certain effects of streptomycin on mycobacteria *in vitro*, Am. Rev. Tuberc., 1946, *54*, 553.

(3) Steenken, W., Jr.: Streptomycin in guinea pig tuberculosis, Am. Rev. Tuberc., 1947, *56*, 382.

(4) Feldman, W. H., Karlson, A. G., and Hinshaw, H. C.: Streptomycin in experimental tuberculosis: The effects in guinea pigs following infection by intravenous inoculation, Am. Rev. Tuberc., 1947, *56*, 346.

(5) Barnwell, J. B., Bunn, P. A., and Walker, A. M.: The effect of streptomycin upon pulmonary tuberculosis: Preliminary report of a coöperative study of 223 patients by the Army, Navy, and Veterans Administration, Am. Rev. Tuberc., 1947, *56*, 485.

(6) Youmans, G. P., and Karlson, A. G.: Streptomycin sensitivity of tubercle bacilli, Am. Rev. Tuberc., 1947, *55*, 529.

(7) Bernstein, S., D'Esopo, N. D., and Steenken, W., Jr.: Streptomycin resistant tubercle bacilli, Am. Rev. Tuberc., 1948, *58*, 344.

(8) WOLINSKY, E., REGINSTER, A., AND STEENKEN, W., JR.: Drug-resistant tubercle bacilli in patients under treatment with streptomycin, Am. Rev. Tuberc., 1948, *58*, 335.

(9) HOWLETT, K. S., JR., AND O'CONNOR, J. B.: Treatment of tuberculosis with streptomycin: Response of certain subacute and chronic types, Am. Rev. Tuberc., 1948, *58*, 139.

(10) STEENKEN, W., JR.: Minutes of Fifth Streptomycin Conference, Veterans Administration, Chicago, Illinois, April, 1948.

(11) WOLINSKY, E., AND STEENKEN, W., JR.: Effect of streptomycin on the tubercle bacillus, Am. Rev. Tuberc., 1947, *55*, 281.

(12) JENKINS, D. E., PECK, W. M., REID, J. J. R., AND WILLIS, H. S.: Effect of streptomycin on early tuberculous lesions, Am. Rev. Tuberc., 1947, *56*, 387.

(13) YEGIAN, D., AND VANDERLINDE, R. J.: A quantitative analysis of the resistance of mycobacteria to streptomycin, J. Bact., 1948, *56*, 177.

(14) BROSIUS, W. L., AND WOODRUFF, C. E.: The effect of sensitivity on the distribution of tubercle bacilli in tuberculosis, Am. Rev. Tuberc., 1944, *50*, 473.

(15) KIRBY, W. M. M.: Bacteriostatic and lytic actions of penicillin on sensitive and resistant staphylococci, J. Clin. Investigation, 1945, *24*, 165.

(16) BIRKELO, C. C., AND BROSIUS, W. L.: Roentgen visualization of pulmonary arterial circulation in autopsy material, Radiology, 1938, *31*, 261.

(17) STEENKEN, W., JR., D'ESOPO, N. D., AND WOLINSKY, E.: Excretion of streptomycin into tuberculous cavities, the pleural space and the tracheobronchial tree, Am. Rev. Tuberc., 1947, *56*, 403.

(18) STEENKEN, W., JR.: Personal Communication.

(19) DUBOS, R. J.: Rapid and submerged growth of mycobacteria in liquid media, Proc. Soc. Exper. Biol. & Med., 1945, *58*, 361.

(20) BALLON, H. C., AND GUERNON, A.: The action of sulfanilamide upon the growth of the tubercle bacillus *in vitro*, J. Thoracic Surg., 1938, *8*, 184.

(21) BALLON, H. C., GUERNON, A., AND SIMON, M. A.: Sulfathiazole in experimental tuberculosis of the guinea pig, Am. Rev. Tuberc., 1942, *45*, 217.

(22) FOLLIS, R. H.: Sulfanilamide, acetylsulfanilamide and sulfapyridine, their effect on *M. Tuberculosis hominis in vitro*, Am. Rev. Tuberc., 1940, *41*, 117.

(23) DEMEREC, M.: Origin of bacterial resistance to antibiotics, J. Bact., 1948, *56*, 63.

(24) VENNESLAND, K., EBERT, R. H., AND BLOCH, R. G.: The demonstration of naturally occurring streptomycin-resistant variants in the human strain of tubercle bacillus H-37Rv, Science, 1947, *106*, 476.

(25) MCDERMOTT, W.: The significance of the finding of tubercle bacilli resistant to streptomycin *in vitro* in the anti-microbial therapy of tuberculosis, Bull. New York Acad. Med., 1948, *24*, 131.

(26) KARLSON, A. G.: Personal communication.

(27) KAHN, M. C.: A developmental cycle of the tubercle bacillus as revealed by single cell studies, Am. Rev. Tuberc., 1929, *20*, 150.

(28) GARDNER, A. D.: Growth and reproduction of B. tuberculosis, J. Path. & Bact., 1929, *32*, 715.

(29) LOEWE, L., AND ATURE-WERBER, E.: Streptomycin dosage schedules for clinical use, Bull. New York Acad. Med., 1947, *23*, 589.

SENSITIVITY OF TUBERCLE BACILLI TO STREPTOMYCIN[1, 2, 3]

The Influence of Various Factors Upon the Emergence of Resistant Strains

KIRBY S. HOWLETT, JR., JOHN B. O'CONNOR, JOSEPH F. SADUSK, JR., WILLIAM E. SWIFT, JR., AND FREDERICK A. BEARDSLEY[4]

(Received for publication January 11, 1949)

INTRODUCTION

The frequent emergence of streptomycin-resistant strains of tubercle bacilli in tuberculous patients treated with streptomycin is now a well known phenomenon (1 to 25). The great importance of these resistant strains in sharply limiting the effectiveness and clinical usefulness of streptomycin is equally well recognized. An increasing number of studies have been stimulated by an interest in possible methods for reducing the incidence of streptomycin-resistant tubercle bacilli and for circumventing the therapeutic obstacles which they create. Of the many variables which might influence the emergence of resistant strains, interest thus far has been largely concentrated upon: (*1*) variations in the daily dose, in the frequency of administration and in the total duration of streptomycin treatment (the regimen employed); (*2*) therapeutic combinations of streptomycin and other drugs. Up to the present time little attention has been given to possible influences inherent in the patient or in the tuberculous disease being treated.

In a detailed report of the study at the Laurel Heights Sanatorium of streptomycin in pulmonary (and bronchopulmonary) tuberculosis (7) which was submitted in February, 1948, analysis of sensitivity data available at the time resulted in the following comments:

> "Among patients on Regimen A or B [1.8 or 2.0 Gm. of streptomycin daily for 120 days] strains requiring 50 γ per cc. or more for inhibition[5] emerged only among those patients with frank caseation or cavitation. . . . It appears from our study that lesions in which caseation and cavitation were prominent were not only least likely to show adequate therapeutic response to streptomycin alone, but were also most likely to produce strains of organisms with significant resistance to the drug."

---

[1] From the Laurel Heights State Tuberculosis Sanatorium, Shelton, Connecticut, and the Department of Internal Medicine, Yale University School of Medicine.

[2] This study is part of the Streptomycin-Tuberculosis Research Project of the American Trudeau Society, Medical Section of the National Tuberculosis Association. Streptomycin used in the earlier portion of the study was generously donated to the Society by the Abbott Laboratories, Eli Lilly and Company, Merck and Company, Inc., Charles Pfizer and Company, Inc., E. R. Squibb and Sons, and the Upjohn Company. Subsequently the study was continued as part of the Comparative Regimens Study of the U. S. Public Health Service.

[3] This study was aided by grants from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service, and from the Fluid Research Fund of Yale University School of Medicine.

[4] With the technical assistance of Mr. John Vadney, Mr. Hilary Morris, Miss Eleanora Falco, and Mr. Adolph Maruschak.

[5] Since the tests used in this study contained no dilution between 10 and 50 γ of streptomycin per cc., this could also be stated as "strains uninhibited by 10 γ per cc. or less."

Subsequently, the response of certain types of subacute and chronic pulmonary tuberculosis treated with streptomycin was the subject of a special report (8). It was observed that resistant strains of tubercle bacilli (uninhibited by 10 γ per cc. of streptomycin) occurred in none of a group of patients with disseminated nodular pulmonary tuberculosis unless cavity was also present. There was a similar complete absence of highly resistant tubercle bacilli in a group of patients, termed "grumblers", who were treated for other types of persistently active but indolent chronic noncavitary pulmonary tuberculosis. Experience in these two groups was in marked contrast to the experience in patients with frankly caseous or cavernous disease in whom the emergence of highly resistant strains had occurred with significant frequency, even when streptomycin was administered for only 42 days. Again, therefore, there appeared to be a very significant relationship between the incidence of streptomycin-resistant tubercle bacilli and the type of tuberculosis treated.

Complete sensitivity data are now available on a considerably larger number of patients treated for various types of pulmonary tuberculosis. The principal purpose of the present report is to survey in detail two relationships, (*1*) between the incidence of streptomycin-resistant tubercle bacilli and the treatment regimen employed; (*2*) between the incidence of streptomycin-resistant tubercle bacilli and the type of pulmonary tuberculosis being treated.

ANALYSIS OF SENSITIVITY DATA

Materials and Methods

Specimens of sputum or gastric contents from streptomycin-treated patients were collected before treatment and at regular intervals thereafter. These specimens were cultured on solid media at the Laurel Heights Sanatorium and the tubercle bacilli thus isolated were then tested for sensitivity. Sensitivity tests on the great majority of the patients in this report were performed at the Yale University School of Medicine by three of the present writers who have reported elsewhere (9, 10) details regarding the method employed. Briefly, the bacilli were first subcultured in a modified Dubos liquid medium and the subculture was used for the inoculation of tubes of the same medium which contained the following dilutions of streptomycin: 0 (control), 0.5, 1.0, 5.0, 10, 50, 100, 500, and 1,000 γ per cc. Sensitivity was determined as the lowest concentration of streptomycin in which no growth could be detected after ten to fourteen days of incubation at 37°C. Very recently the sensitivity tests for the Laurel Heights study have been performed, instead, at the Trudeau Laboratory by Steenken (11) and his associates, using solid medium containing 0 (control), 1.0, 10, and 100 γ of streptomycin per cc. Thus far, however, complete sensitivity data from this source have become available on only a very few patients.

For purposes of this analysis (and for consistency with other reports submitted on patients in the Laurel Heights study) the streptomycin treatment regimens which have been explored are designated as follows:

Regimen A: 1.8 Gm. daily for 120 days.

Regimen B: 2.0 Gm. daily for 120 days.

Regimen C: 1.0 Gm. daily for 42 days, later revised to 20 mg. per kg. of body weight daily for 45 days.

Regimen III: 40 mg. per kg. of body weight daily, two consecutive days per week (with no streptomycin on the other days of the week) for a total of 13 weeks.

Regimen IV: 20 mg. per kg. of body weight daily for 90 days.

Because of the close similarity of Regimens A and B, patients on these two regimens are

reported together in one group. Regimens III and IV have been used in only a few patients and complete sensitivity data are not yet available on enough of them to justify their inclusion in the analysis by regimens (table 1). All patients with adequate sensitivity data (whatever the regimen employed) have, however, been included in the analysis by type of disease (table 2).

In 8 patients in table 2, sensitivity data were obtained from the Trudeau Laboratory where the test contained no dilution of streptomycin between 1.0 and 10 γ per cc. Pretreatment specimens in all 8 showed growth in 1.0 γ per cc.; 7 of the 8 showed no growth in 10 γ per cc.; one of the 8 showed two colonies in 10 γ per cc., none in 100 γ per cc. In the remaining patients in table 2 and in all patients in table 1, sensitivity data were obtained from the Yale University School of Medicine. On the basis of the test in liquid medium employed there, pretreatment cultures were regularly sensitive to either 0.5 or 1.0 γ per cc. except in 5 patients. In each of these 5 instances a pretreatment reading of 5.0 γ was obtained, although cultures from all 5 patients also had readings of 0.5 or 1.0 γ on subsequent tests.

Unless otherwise specified, all the data which follow are based on at least one or more cultures obtained at or after the end of treatment (as well as during treatment). When the last positive culture during treatment was still sensitive, but sputum and gastric contents became consistently negative prior to the end of treatment and remained so, the result has been classed as indeterminate. This has been done to avoid finally classifying any patient's tubercle bacilli as highly sensitive or intermediate unless the effect upon sensitivity of the entire course of streptomycin is known. This report is concerned, however, only with the effect of the primary course of streptomycin upon sensitivity. A few patients have subsequently been retreated, but data on the effect of retreatment upon sensitivity are too few for analysis, and such data have been excluded from the tables which follow.

In tables 1 and 2 the strains of *M. tuberculosis* isolated from the patients are classified as follows:

1. Highly resistant, *i.e.*, requiring more than 10 γ per cc. to inhibit growth[6];
2. Intermediate, which is subdivided into (a) cultures inhibited by 10 γ per cc. but not by 5, and (b) cultures inhibited by 5 γ but not by 1.0;
3. Highly sensitive, *i.e.*, cultures inhibited by 0.5 or 1.0 γ per cc.;
4. Indeterminate, *i.e.*, no positive culture could be obtained after the end of treatment but all cultures during treatment were inhibited by 5.0 γ per cc. (1 patient) or less (all other patients in this group).

Analysis by Regimens

Complete sensitivity data are now available on 28 patients treated on Regimens A or B and on 47 patients treated on Regimen C. These data are tabulated in table 1.

In 4 patients on Regimen C, strains resistant to 1,000 γ per cc. eventually emerged in spite of the fact that cultures at the end of treatment were still sensitive to 5.0 γ per cc. or less. In these patients the highly resistant strains first appeared in specimens obtained several weeks after the completion of treatment. Amberson and Stearns (12) and Wolinsky, Reginster and Steenken (13) have also reported cases treated for 42 days in which increasingly resistant strains have been demonstrated after cessation of treatment.

In view of the relatively small number of cases reported, it was thought best not to attempt further subdivision in table 1. It is of interest, however, that

[6] On the basis of the tests employed in this study, cultures which grew in 10 γ per cc. required 50 γ per cc. or more for inhibition. Actually the majority of patients classed as resistant eventually discharged bacilli which were uninhibited by 1,000 γ per cc.

among the 28 patients treated on Regimens A or B, frank caseation or cavity was judged to have been present prior to treatment in 18 (64 per cent) and absent in 10 (36 per cent). Among the 47 patients treated on Regimen C, frank caseation or cavity was judged to have been present prior to treatment in 26 (55 per cent), absent in 21 (45 per cent). There is, therefore, no apparent basis for attributing the failure of Regimen C to reduce the incidence of streptomycin-resistant tubercle bacilli to a higher proportion of unfavorable cases in this group.

Analysis by Types of Pulmonary Tuberculosis

For this analysis, patients treated on various regimens have been divided into two broad categories according to the pulmonary disease present prior to treatment. The division has been based almost entirely on the interpretation of pretreatment roentgenograms, although this interpretation was made with a knowl-

TABLE 1

Correlation of sensitivity data to the treatment regimen employed

SENSITIVITY OF TUBERCLE BACILLI TO STREPTOMYCIN AT OR AFTER END OF TREATMENT	REGIMEN A OR B PATIENTS AND PER CENT		REGIMEN C PATIENTS AND PER CENT	
Resistant (> 10 γ)	8	(29%)	13	(28%)
Intermediate: a) 10 γ	5 (18%)	7 (25%)	2 (4%)	10 (21%)
b) 5 γ	2 (7%)		8 (17%)	
Sensitive (0.5 or 1.0 γ)	7	(25%)	19	(40%)
Indeterminate*	6	(21%)	5	(11%)
Total	28	(100%)	47	(100%)

* Last positive culture during treatment sensitive to 5.0 γ (1 patient) or to 0.5 or 1.0 γ (10 patients) but with cultures consistently negative prior to the end of treatment and subsequently.

edge of collateral clinical and laboratory findings. Planigrams were used freely whenever the presence of cavity was considered questionable on examination of the conventional films.

On this basis, frank caseation or cavity was judged to have been present prior to treatment in 56 patients, while in 31 patients there were no lesions deemed to be frankly caseous or cavernous. Sensitivity data for the two groups are recorded in table 2.

Of the 56 patients with caseation or cavity recorded in table 2: 18 (32 per cent) were treated on Regimen A or B, 26 (47 per cent) were treated on Regimen C, 4 (7 per cent) were treated on Regimen III, and 8 (14 per cent) were treated on Regimen IV. Of the 31 patients without caseation or cavity recorded in table 2: 10 (32 per cent) were treated on Regimen A or B, and 21 (68 per cent) were treated on Regimen C[7]. In the group with caseation or cavity, therefore, a

[7] In a few patients there were slight deviations in either daily dose or duration from the exact regimens listed, but in no instance was such deviation more than minor. When toxic manifestations of streptomycin caused treatment to be terminated within less than 42 days, the patient has been excluded from this report.

somewhat larger proportion of patients received streptomycin for periods of time longer than 45 days than was the case in the group without caseation or cavity. It is possible that the higher incidence of resistant strains in the caseous-cavernous group may be attributable, in part, to this fact. On further analysis, however, it is apparent that this is not the principal factor involved.

All of the patients on Regimens III and IV were patients with gross cavitation, and most of them were deliberately chosen for this reason in order to explore the relative effect of these regimens upon resistance. In 9 of these patients sensitivity after 45 days of treatment (as well as after the end of treatment) is known, and in 7 of the 9 (including all 4 patients on Regimen III) highly resistant strains were demonstrated to have been present in large numbers by the end of 45 days. When these patients are added to the 13 patients on Regimen C, who produced highly resistant strains, there is a total of 20 patients (36 per cent of the entire group of patients with caseation or cavity) in whom resistant strains are known

TABLE 2

Correlation of sensitivity data to the type of pulmonary tuberculosis present prior to treatment

SENSITIVITY OF TUBERCLE BACILLI TO STREPTOMYCIN AT OR AFTER END OF TREATMENT	CASEATION OR CAVITY PRESENT PATIENTS AND PER CENT		NO CASEATION OR CAVITY PATIENTS AND PER CENT	
Resistant (> 10 γ)		33 (59%)		0
Intermediate: a) 10 γ	4 (7%)	11 (20%)	4 (13%)	6 (19%)
b) 5 γ	7 (13%)		2 (6%)	
Sensitive (0.5 or 1.0 γ)		9 (16%)		17 (55%)
Indeterminate*		3 (5%)		8 (26%)
Total		56 (100%)		31 (100%)

* See footnote, Table 1.

to have emerged as the result of *45 days or less* of streptomycin treatment. This figure (as well as the 59 per cent total incidence of resistant strains in the caseous-cavernous group) is in marked contrast to the failure thus far of highly resistant strains to emerge in force in any patient *without* frank caseation or cavity. Evidence in this study that the type of tuberculosis being treated greatly influenced the incidence of streptomycin-resistant tubercle bacilli, regardless of the treatment regimen employed, appears, therefore, to be unequivocal.

DISCUSSION

General Considerations

It is apparent from several reports (11, 13, 14, 15, 16, 22, 24, 25) that the specific sensitivity data obtained in a given study are considerably influenced by variables in the method of testing, such as the culture medium employed, the size of the inoculum, the incubation time before the test is read, et cetera. Especially noteworthy is the fact that tests in a liquid medium yield results which indicate merely the presence or absence in the inoculum of drug-resistant organ-

isms in sufficient numbers to support growth. An unknown proportion of the patient's bacilli may still be far more sensitive than such a reading indicates. These variables lead to reservations in the interpretation of sensitivity data now available, especially when results from one laboratory are compared with those from another in which different methods were employed. For this report, however, all the tests recorded in table 1 and all but a very few of those recorded in table 2 were performed in the same laboratory by the same method. Moreover, the sensitivity level reported for each patient's bacilli is generally based on repeated tests performed both during and after treatment. Thus the influence of technical factors upon the *comparative* data in the groups reported here has been minimized.

It is difficult to establish an absolutely uniform relationship between *in vitro* resistance and *in vivo* resistance. This is not surprising when one considers not only the variables listed in the preceding paragraph but also the variables in the spontaneous behavior of pulmonary tuberculosis which have always made accurate evaluation of a single factor so difficult. Nevertheless, as Walker (17) has said (in summarizing data from the joint streptomycin study of the Army, Navy, and Veterans Administration), there is an accumulating mass of evidence, no one piece decisive in itself, which indicates that the observation of *in vitro* resistance has very real clinical significance. Experience in the Laurel Heights study strongly supports this conclusion. To say the least, streptomycin is very unlikely to affect a patient's clinical course favorably, once resistant strains of tubercle bacilli appear in sufficient numbers to be demonstrated by the tests in liquid media which have been generally employed thus far in the study of this phenomenon.

The exact level at which *in vitro* resistance become clinically significant is far more dubious. On the basis of both theoretical considerations and practical experience (11, 17, 18, 19, 20) there appears to be good reason for classifying as significantly resistant those strains of tubercle bacilli which grow readily in culture media containing 10 γ of streptomycin per cc. and which require 50 γ per cc. or more to inhibit growth. Certainly bacilli which do not grow in concentrations of 1.0 γ per cc. or less must be considered highly sensitive. Patients who discharge bacilli with *in vitro* sensitivity between these two extremes have been tabulated separately in table 2.

The total incidence of streptomycin-resistant strains of tubercle bacilli encountered in this study (28 per cent of all patients included in table 1, 38 per cent of all patients included in table 2) is considerably lower than that reported from certain other sources (11, 17, 20, 21). It is possible that this difference results in part from the variables already discussed. It is undoubtedly a consequence, in part, to a difference in the method of reporting. In this report, the incidence of drug-resistant strains is expressed as a percentage of the *total* number of patients in a given category; in other reports the incidence has usually been expressed as a percentage of the number of positive cultures (or of patients producing positive cultures) at a specified interval of time after the start of treatment. It is believed, however, that a difference in case material is the most im-

portant factor to account for the lower incidence of resistant strains in this study. The study has included a considerable proportion of patients without frank caseation or cavity, most of whom were treated for relatively indolent types of pulmonary tuberculosis. In such patients the incidence of resistant strains is shown to be extremely low (table 2).

The Influence of the Treatment Regimen Upon Sensitivity of M. Tuberculosis to Streptomycin

On the basis of experience in the joint Army, Navy, and Veterans Administration study (17) it was concluded that changes in the daily dose of streptomycin from 2.0 Gm. to 1.0 Gm. and, subsequently, even to 0.5 Gm. resulted in only minor differences in the incidence of streptomycin-resistant strains of tubercle bacilli. On all three regimens, resistant strains appeared after 30 to 45 days of treatment in an increasing number of patients until, after 120 days, about 70 per cent of positive cultures grew readily in concentrations of more than 10 γ per cc. The curves from this and other studies have suggested that the emergence of resistant strains was influenced far more by the duration of treatment than by daily dose of streptomycin, and that the percentage of resistant cultures was consistently low prior to approximately the sixth week of treatment. It was largely for this reason that a number of investigators have chosen to try shorter regimens, for example 42 or 45 days, in the hope that the resulting incidence of resistant strains would be materially reduced.

The number of cases in table 1 is undoubtedly too small to permit definitive conclusions from this study alone. It is apparent, however, that the change from the 120 day to the 42 day regimen has produced little change in the incidence of resistant strains encountered in this particular study. This is partly a consequence of the fact that increasingly resistant strains have appeared in some patients on the 42 day regimen after treatment was discontinued. If only those patients in table 1 known to have all cultures highly sensitive are considered, then Regimen C does have a distinct advantage. Even this advantage becomes slight, however, if patients whose sputum cultures became consistently negative during treatment (but whose last positive culture was highly sensitive) are included in the sensitive group. At the very least it must be concluded that reduction in the duration of streptomycin treatment from 120 days to 42 (or 45) days has been very much less successful than had been hoped in reducing the incidence of resistant strains of tubercle bacilli.

It is of incidental interest that there is limited evidence in the Laurel Heights study to suggest that, if tubercle bacilli are sensitive to streptomycin when sputum conversion occurs, they are likely to remain so. In addition to the patients whose bacilli must still be classed as indeterminate in tables 1 and 2, there were 8 other patients whose sputum and gastric contents became consistently negative for tubercle bacilli during treatment and whose cultures, prior to becoming negative, had all been sensitive to 0.5 or 1 γ. However, each of those 8 patients subsequently produced at least one or more positive specimens which permitted sensitivity again to be determined. These positive specimens occurred

from two to ten months after treatment had been completed. In every instance tubercle bacilli in the posttreatment specimen were sensitive to 10 γ per cc. or less, and in 6 of the 8 the bacilli were still sensitive to 0.5 or 1.0 γ. This experience is, however, different from that of Fisher (11) who found resistant strains in posttreatment specimens from approximately one-half of his patients who had obtained temporary bacteriologic conversion but had had recurrence of positive sputum three to twelve months later. (Fisher does not state whether or not resistant strains had already been observed in any of these patients prior to temporary sputum conversion.) At the present time one is compelled to regard sensitivity as indeterminate until the effect of the entire course of streptomycin treatment is definitely known.

The Influence of the Type of Pulmonary Tuberculosis upon Sensitivity of M. Tuberculosis to Streptomycin

Although the number of patients in table 2 is not large, the relationship between the type of tuberculosis being treated and the incidence of resistant tubercle bacilli appears far too close to be the result merely of chance. The patients in table 2 have been divided only into two broad categories of disease. Further subdivision would result in such small groups that the significance of any correlation observed might be questionable. It should be stated, however, that most (though not all) of the patients who had no frankly caseous or cavernous lesions were patients with the types of subacute or chronic pulmonary tuberculosis mentioned previously, namely those with disseminated nodular pulmonary tuberculosis and those termed "grumblers" (8). In these patients, the tuberculosis, while obviously active, was generally rather indolent in character. In contrast, the majority of the patients with frank caseation or cavity were treated for disease of much greater activity. In a considerable number, the tuberculosis was very active and rapidly progressive when treatment was started. It is believed that the activity of the tuberculosis was also a factor pertinent to the higher incidence of resistant strains in the caseous-cavernous group.

During the early experience with streptomycin in Great Britain, which was recently reported (21), treatment of pulmonary tuberculosis was confined to acute progressive tuberculosis of "bronchopneumonic" type, with or without cavity, in patients between the ages of fifteen and thirty. On the basis of sensitivity data from 41 such patients, it was concluded that there is a possibility of a relationship between the severity of the patients' clinical condition (high fever, large or multiple cavities) just prior to treatment and the development of high degrees of streptomycin-resistance. Also, Crofton and Mitchison (22), who participated in this cooperative British study, have analyzed data from 13 of their own patients. They found that resistant strains occurred earlier and that resistance reached higher levels in patients who did poorly during the early stages of streptomycin treatment than in patients who responded promptly and favorably.

In the Laurel Heights study, patients severely ill with the acute pneumonic forms of pulmonary tuberculosis have been relatively few. Included in table 2

are, however, 13 patients who were very ill with extensive progressive tuberculosis and high fever when streptomycin treatment was started. All these patients had extensive cavitation with highly active collateral disease, dense consolidations of at least lobar extent, or both. Among these 13 patients, resistant strains of tubercle bacilli emerged in 12. It is of further interest that in this same group there were 7 patients whose clinical response to streptomycin was negligible even during the first weeks of treatment. This experience was rarely encountered in other patients who were obviously ill clinically, but less severely so. The one severely ill patient who failed to discharge resistant strains of bacilli showed immediate and excellent clinical response to streptomycin treatment and eventually obtained almost complete resolution of an extensive tuberculous pneumonia. His sputum became consistently negative for tubercle bacilli after the third month of treatment and the sensitivity readings on all previous specimens had been 1.0 γ or less. In retrospect it is believed that caseation in this case was actually far less extensive than it was originally judged to be. In many patients, highly active tuberculosis, severe symptoms, extensive caseation or cavity, and poor response to treatment are such closely interrelated phenomena that it becomes difficult to analyze them separately. The experiences mentioned above suggest strongly that treatment of caseous or cavernous pulmonary tuberculosis with streptomycin for periods of 42 days or longer is, indeed, especially likely to result in the emergence of resistant strains of tubercle bacilli when the patient is severely ill with extensive and highly active disease. When, in addition, such a patient shows poor initial response to treatment, the eventual occurrence of resistant strains would seem to be almost certain.

Highly resistant strains eventually occurred, however, in several afebrile patients with chronic cavity and with only slowly progressive or chronically active collateral disease. Resistant strains also occurred in a considerable number of patients whose response to treatment, initially, was very prompt and very favorable. On the other hand, strains of bacilli uninhibited by 10 γ of streptomycin per cc. or less have occurred in none of several febrile patients treated promptly for acute exudative spreads of tuberculosis, but in whom frank caseation or cavity could not be detected. It appears from this study, therefore, that the presence or absence of frank caseation or cavity bears a far more consistent relationship to the emergence of resistant strains of tubercle bacilli than does the acuteness of the tuberculosis, the severity of symptoms, or the initial clinical response to treatment, when these factors are considered separately.

It cannot be inferred, however, that patients without frank caseation or demonstrable cavity in pretreatment roentgenograms are necessarily immune to the hazard of the emergence of drug-resistant strains of bacilli (table 2). The number of patients in this category is small, and, although highly resistant strains have not yet emerged in this particular group, strains less sensitive than those present prior to treatment have appeared. There is little reason to doubt that highly resistant strains also will eventually be encountered among patients of this type, though it is expected that the percentage will be small. Furthermore, it is obviously impossible in many instances to exclude completely the

presence of caseation or cavity on the basis of roentgenograms. In fact, even when *frank* caseation or cavity could not be demonstrated in the patients reported here, it is extremely likely that small foci of caseation often existed. Also, since tubercle bacilli were present in the sputum or gastric contents of all patients, it must be assumed that some degree of ulceration was present. In other words, it is well recognized that interpretations based largely on roentgenograms are bound to fall far short of strict pathological accuracy. Nevertheless, such interpretations have been made by phthisiologists long before the advent of streptomycin, and have long been used as an important guide to the management of patients. The practical value of such interpretations can scarcely be questioned.

The presence or absence of *frank* caseation or cavity, as judged from roentgenograms, has already been shown to influence greatly the therapeutic results obtained from streptomycin (7, 11, 17, 19, 23). It is not unreasonable that the incidence of resistant tubercle bacilli should also be influenced. It is in such lesions (and especially in the highly active and progressive ones) that tubercle bacilli are likely to be most numerous, that multiplication is likely to be most rapid, and that suppression by streptomycin is likely to be most limited.

In the present study the classification of patients on this basis actually presented little difficulty, although, naturally, borderline cases arose. The vast majority of patients judged to have caseous disease also had definite and clearly demonstrable cavity. In fact only 2 patients were placed in this category on the basis of caseation alone. In instances where small cavity was suspected but could not be definitely outlined, cavity was judged not to be present for purposes of classification.

Follow-up of patients treated at the Laurel Heights Sanatorium (7) has strengthened the conclusion that, when treatment by bed rest has failed, streptomycin alone is unlikely to produce sustained arrest of frankly caseous or cavernous pulmonary tuberculosis. In such cases, optimum results can be achieved only by the integration of streptomycin with appropriate forms of collapse therapy, resection, or other surgery. The observation that strains of tubercle bacilli resistant to streptomycin are especially likely to emerge, and that they often emerge rapidly in patients with frank caseation or cavity, further emphasizes the importance of an overall plan of therapy in such cases. It is only in this way that appropriate collapse therapy or other surgery can be applied promptly, early in the course of streptomycin therapy, before resistant strains emerge and prevent further benefit from the drug.

SUMMARY

1. Data on the sensitivity of tubercle bacilli to streptomycin have been presented in a series of patients treated with streptomycin for pulmonary tuberculosis. All were treated for 42 days or longer. Among these patients, the risk of streptomycin resistance proved to be related far more closely to the type of tuberculosis being treated than to the particular treatment regimen chosen. The presence or absence of frank caseation or cavity appeared to be the factor which most consistently influenced the liability to resistant strains.

2. In a group of 28 patients treated on a regimen of 1.8 or 2.0 Gm. of streptomycin daily for 120 days, resistant strains of tubercle bacilli eventually occurred in 8 (29 per cent). In a group of 47 patients treated on a regimen of 1.0 Gm. of streptomycin daily for 42 days (later revised to 20 mg. per kg. of body weight daily for 45 days), resistant strains eventually occurred in 13 (28 per cent). The number of patients in these two groups is too small to demonstrate in a definitive manner the relative effect of these regimens upon resistance. It is concluded, however, that a reduction in the duration of streptomycin treatment from 120 days to 42 (or 45) days has been considerably less successful than had been hoped in reducing the incidence of resistant tubercle bacilli.

3. A very impressive correlation has been observed between the incidence of resistant tubercle bacilli and the type of tuberculosis being treated. Resistant strains have emerged in a high proportion of patients who had frank caseation or cavity in pretreatment roentgenograms. In a group of 56 such patients, treated on the several regimens employed in this study, strains uninhibited by 10 γ of streptomycin per cc. of culture medium eventually occurred in 33 (59 per cent). In contrast (and regardless of the treatment regimen employed), strains uninhibited by 10 γ per cc. or less have occurred thus far in none of 31 patients who had no frankly caseous or cavernous lesions prior to treatment.

SUMARIO

Sensibilidad de los Bacilos Tuberculosos a la Estreptomicina. El Influjo de Varios Factores sobre la Emergencia de Cepas Resistentes.

1. Los datos presentados sobre la sensibilidad de los bacilos tuberculosos a la estreptomicina básanse en una serie de enfermos tratados con dicha droga por tuberculosis pulmonar. Todos ellos fueron tratados por espacio de 42 días o más. Entre ellos, el riesgo de la aparición de estreptomicinorresistencia resultó hallarse mucho más relacionado con la forma de tuberculosis en tratamiento que con el régimen terapéutico escogido. La presencia o ausencia de caseación o cavernas bien definidas pareció ser el factor que afectó más poderosamente la propensión al desarrollo de cepas resistentes.

2. En un grupo de 28 enfermos tratados con un régimen de 1.3 a 2.0 Gm. diarios de estreptomicina durante 120 días, se presentaron con el tiempo cepas resistentes de bacilos tuberculosos en 8 (29 por ciento). En un grupo de 47 tratados con un régimen de 1.0 Gm. diario de estreptomicina durante 42 días (modificado luego a 20 mg. diarios por kg. de peso durante 45 días), aparecieron con el tiempo cepas resistentes en 13 (28 por ciento). El número de enfermos en esos dos grupos es demasiado pequeño para demostrar definitivamente el relativo efecto de esos dos régimenes sobre la resistencia. Dedúcese, sin embargo, que la disminución de la duración de la estreptomicinoterapia de 120 a 42 (o 45) días ha surtido mucho menos efecto que lo esperado en lo tocante a reducir la incidencia de bacilos tuberculosos resistentes.

3. Observóse una correlación muy importante entre la incidencia de cepas tuberculosas resistentes y la forma de tuberculosis en tratamiento, apareciendo aquellas en una elevada proporción de los enfermos que mostraron manifiesta

caseación o caverna en las radiografías anteriores al tratamiento. En un grupo de 56 de estos enfermos, tratados con los varios régimenes empleados en el estudio actual, surgieron con el tiempo cepas que no inhibían 10 γ de estreptomicina por cc. de medio de cultivo en 33 (59 por ciento). En contraposición (e independiente del régimen terapéutico empleado), hasta la fecha no se han observado cepas que no inhibieran 10 γ por cc. o menos en ninguno de los 31 enfermos que no tenían lesiones francamente caseosas o cavernosas antes del tratamiento.

REFERENCES

(1) Youmans, G. P., Williston, E. H., Feldman, W. H., and Hinshaw, H. C.: Increase in resistance of tubercle bacilli to streptomycin, Proc. Staff Meet., Mayo Clin., 1946, *21*, 126.

(2) The Committee on Chemotherapeutics and Other Agents, National Research Council: Keefer, C. S., *et al.*: Streptomycin in the treatment of infections, J. A. M. A., 1946, *132*, 70.

(3) Report of the Committee on Therapy, Hinshaw, H. C., Chairman, Am. Rev. Tuberc., 1946, *54*, 439.

(4) Youmans, G. P., and Karlson, A. G.: Streptomycin sensitivity of tubercle bacilli: Studies on recently isolated tubercle bacilli and the development of resistance to streptomycin *in vivo*, Am. Rev. Tuberc., 1947, *55*, 529.

(5) The effects of streptomycin on tuberculosis in man: Preliminary statement: Office of Chief Medical Director, Veterans Administration, the Surgeon General of the Army and the the Surgeon General of the Navy, J. A. M. A., 1947, *135*, 634.

(6) Muschenheim, C., McDermott, W., Hadley, S. J., Hull-Smith, H., and Tracy, A.: Streptomycin in the treatment of tuberculosis in humans: II. Pulmonary tuberculosis, Ann. Int. Med., 1947, *27*, 989.

(7) Howlett, K. S., Jr., and O'Connor, J. B.: Treatment of tuberculosis with streptomycin: A clinical report, in Streptomycin in the Treatment of Tuberculosis, National Tuberculosis Association, New York (to be published).

(8) Howlett, K. S., Jr., and O'Connor, J. B.: Treatment of tuberculosis with streptomycin: Response of certain subacute and chronic types, Am. Rev. Tuberc., 1948, *58*, 139.

(9) Sadusk, J. F., Jr., and Swift, W. E., Jr.: Sensitivity of the tubercle bacillus to streptomycin before and during specific therapy, J. Clin. Investigation, 1948, *27*, 278.

(10) Swift, W. E., Jr., and Beardsley, F. A., Jr.: The effect of a 42 day course of treatment on the sensitivity of the tubercle bacillus to streptomycin (to be published).

(11) Minutes of the Fifth Streptomycin Conference, Veterans Administration, April, 1948, Chicago, Ill.

(12) Amberson, J. B., and Stearns, W. H.: Streptomycin in the treatment of tuberculosis, Ann. Int. Med., 1948, *29*, 221.

(13) Wolinsky, E., Reginster, A., and Steenken, W., Jr.: Drug-resistant tubercle bacilli in patients under treatment with streptomycin, Am. Rev. Tuberc., 1948, *58*, 335.

(14) Pyle, M. M.: Relative numbers of resistant tubercle bacilli in sputa of patients before and during treatment with streptomycin, Proc. Staff Meet., Mayo Clin., 1947, *21*, 465.

(15) Fisher, M. W.: Streptomycin resistant tubercle bacilli, Am. Rev. Tuberc., 1948, *57*, 53.

(16) Fisher, M. W.: Sensitivity of tubercle bacilli to streptomycin: An *in vitro* study of some factors affecting results in various test media, Am. Rev. Tuberc., 1948, *57*, 58.

(17) Streptomycin Committee, Veterans Administration: Streptomycin in the treatment of tuberculosis: Current status, Report to the Council on Pharmacy and Chemistry, J. A. M. A., 1948, *138*, 584.

(18) Steenken, W., Jr., and Wolinsky, E.: Streptomycin in experimental tuberculosis: II. Response in guinea pigs infected with strains of varying degrees of streptomycin resistance, Am. Rev. Tuberc., 1948, *58*, 353.

(19) D'Esopo, N. D., and Steinhaus, J. E.: Streptomycin therapy: With special reference to pulmonary tuberculosis, Am. Rev. Tuberc., 1947, *56*, 589.

(20) Bernstein, S., D'Esopo, N. D., and Steenken, W., Jr.: Streptomycin resistant tubercle bacilli: Incidence in patients treated with streptomycin, Am. Rev. Tuberc., 1948, *58*, 344.

(21) Streptomycin in Tuberculosis Trials Committee, British Medical Research Council: Streptomycin treatment of pulmonary tuberculosis, Brit. M. J., 1948, *2*, 769.

(22) Crofton, J., and Mitchison, D. A.: Streptomycin resistance in pulmonary tuberculosis, Brit. M. J., 1948, *2*, 1009.

(23) Levine, E. R., Klein, W. S., and Froman, A.: Streptomycin as adjunct in treatment of acute pulmonary tuberculosis, J. A. M. A., 1948, *138*, 808.

(24) Youmans, G. P., and Williston, E. H.: Streptomycin resistant variants obtained from recently isolated cultures of tubercle bacilli, Proc. Soc. Exper. Biol. & Med., 1948, *68*, 458.

(25) Yegian, D., and Vanderlinde, R. J.: A quantitative analysis of resistance of mycobacteria to streptomycin, J. Bact., 1948, *54*, 191.

COEXISTING DISSEMINATED COCCIDIOIDOMYCOSIS AND TUBERCULOSIS[1, 2]

Report of a Case

GEORGE M. FIRESTONE AND ELLIS S. BENSON

(Received for publication September 21, 1948)

INTRODUCTION

Coccidioidomycosis has become a well recognized and clearly defined disease entity and many excellent studies have been made of its epidemiology, pathogenesis, clinical manifestations, and pathology (3, 8, 11). Although the parallelism of coccidioidomycosis and tuberculosis has been well studied (9), there have been only five reports (1, 4, 5, 6, 10) of the coexistence of the two diseases in the same individual. The present report is concerned with a sixth case, which is apparently the first observation of the association of *disseminated* coccidioidomycosis with tuberculosis and in which the dual etiology was proved both by culture and by animal inoculation. It is believed that this case manifests many epidemiologic, therapeutic, and pathologic features of unusual interest.

CASE REPORT

Clinical Course

The patient was a 34 year old white single enlisted man who had been well, except for a chronic "cigarette cough" until early August, 1942, when he was stationed at an Army Camp at Compton, California, some twenty miles south of Los Angeles. He had been stationed at Compton less than one month prior to the onset of symptoms, but had resided in San Diego, California, from November, 1941 to June, 1942 Initial symptoms consisted of a stiffness and soreness in the right anterior cervical region. In mid-August, because of this pain, a right lower tooth was pulled. Toward the end of August he noticed a gradual increase in size of the anterior cervical nodes, especially the right. This was associated with an aching in his neck which was aggravated by dorsiflexion and which kept him awake at night. During the month of August his cough increased in severity, and he developed general malaise, anorexia, and dysphagia.

The patient's past health had been excellent. He had measles and mumps in childhood, and lobar pneumonia at age 16. There is no history of tuberculosis or other chronic disease in himself or his family.

On admission to the 73rd Evacuation Hospital, on September 3, 1942, the patient appeared to be a chronically ill malnourished individual. He had a small superficial nodule with intact dermis and surrounding erythema on the forehead and another over the left scapula. There was a 3 cm. tender hard mass anterior to the right sternocleidomastoid muscle, as well as somewhat tender nodes varying in size from "shotty" to "pigeon egg" in both axillae and right and left anterior and posterior cervical chains. Severe pyorrhea was present. The remainder of the physical examination was negative.

---

[1] From the Tuberculosis and Pathology Services of the Minneapolis Veterans Administration Hospital and the Departments of Medicine and Pathology of the University of Minnesota Medical School.

[2] Published with the permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the author.

On admission the hemogram showed 3.7 million red blood cells per cu. mm., 80 per cent hemoglobin, 16,150 white blood cells per cu. mm., 60 per cent neutrophils, 10 per cent lymphocytes, 1 per cent basophils, 22 per cent eosinophils, and 7 per cent monocytes. The urinalysis was normal except for one plus albumin. The sputum was negative for acid-fast bacilli.

A chest roentgenogram showed metallic fragments present in the shoulder girdle area, at the site of an old bullet wound. There was also a soft 1 cm. infiltration in the right first interspace and two smaller adjacent infiltrations, "characteristic of tuberculosis." Examination of material obtained by biopsy of a lymph node and of one of the skin nodules revealed a "mycotic granuloma due to *Coccidioides immitis*."

Throughout the period spent at the 73rd Evacuation Hospital, the patient displayed an irregular fever with frequent elevations to 100° or 101° F. In September, 1942 a new skin nodule developed on the occiput and the old nodules were seen to be ulcerating and fungating.

The patient was transferred to Hoff General Hospital on September 27, 1942. His weakness and weight loss had progressed and he suffered from occasional spells of shortness of breath. His cough had become productive and he occasionally produced blood-streaked sputum. He first noticed a mild constipation at this time, which continued throughout his illness. There were no genito-urinary symptoms or signs.

Physical examination at this time revealed dirty ulcers, about 1.5 cm. in diameter, with raised red margins on the forehead, occiput and scapula. Enlarged nodes were found in the posterior cervical triangles, the left anterior cervical chain, and in the left axilla. The blood pressure was 110/74 mm. of mercury.

The hemogram remained essentially the same, with moderate leukocytosis and eosinophilia. The sedimentation rate varied from 72 to 117 mm. per hour. Urinalyses revealed a trace to one plus albuminuria as the only abnormality. Roentgenograms disclosed an increase in the infiltration in the right apex as well as new soft infiltrations in the left apex and left second interspace. Increased hilar shadows were also present. Subsequent roentgenograms showed progressive clearing so that by December, 1942, no active lesions were present. Roentgenograms of the skull and shoulder regions were negative.

Examination of material obtained by biopsy of the skin ulcer again showed a granulomatous lesion containing spherules of *Coccidioides immitis*. Three sputum examinations for acid-fast organisms were negative, but *Coccidioides immitis* was reported present in the sputum. Skin tests with coccidioidin in dilutions of 1:1,000, 1:100, and 1:10 were all negative. Complement fixation tests gave a one plus reaction at 1:64 dilution, and a positive precipitin test in a dilution of 1:40.[2] Complement fixation tests for *Blastomyces*, performed by the Department of Medicine, Duke University School of Medicine, were negative. Material from the forehead lesion sent to Dr. C. E. Smith of Stanford University School of Medicine, cultured on Sabouraud's agar, blood agar, and a special differential *Coccidioides* medium, grew a white cottony fungus which was injected into two mice. Both mice died and typical double-contoured endosporulating spherules were found in the lungs These were cultured a second time and again produced a cottony white fungus. No *Blastomyces* were found.

The patient's course in Hoff General Hospital was at first irregularly febrile, with frequent daily elevations of temperature to 100 to 101° F. Therapeutic trials of sulfathiazole were made on two occasions, but were discontinued each time because of fever and dermatitis. Fever therapy with typhoid vaccine was tried without attaining a significant temperature response. Malaria fever therapy was then instituted, during which the patient developed seven chills with no change in the clinical course. However, the total length of time in which the temperature was over 102° F. was less than twenty hours. Also during

[2] All serological tests, unless otherwise indicated, have been performed by Dr. C. E. Smith, of the Stanford University Medical School, Department of Public Health, and the Commission on Acute Respiratory Diseases of the Army Epidemiological Board.

this period the local skin lesions were excised and treated with roentgen therapy. It was noted that gradually the old skin lesions healed completely although a new papule developed on the ramus of the jaw. The lesions demonstrable on the chest roentgenograms cleared and the temperature gradually became normal.

On January 16, 1943, the patient was transferred to McCloskey General Hospital where, upon admission, it was noted that he felt well, had no cough and no fever. Examination disclosed the well healed scars of the old lesions on the forehead, scapula, occiput, and sternum, with an active crusted lesion present on the ramus of the jaw. Otherwise the physical examination revealed no relevant abnormalities.

Roentgenograms taken at the time of admission showed pleural thickening in the right apex and an infiltration behind the right clavicle, which did not change on subsequent examinations through July 10, 1944. The apex of the left lung was clear. Films of the cervical spine were negative except for the presence of hypertrophic changes about the vertebral bodies. There was no essential change in the red cell count or hemoglobin. The white blood cell count ranged between 5,950 and 9,000 per cu.mm. with 7 to 21 per cent eosinophils. A skin test for tuberculosis using Purified Protein Derivative (first strength) was positive in May, 1943, but thirteen separate sputum examinations for acid-fast bacilli were negative. Except for one positive culture in March, sputum cultures for *Coccidioides immitis* were all negative during 1943. One feces culture for *Coccidioides* was reported positive in February, 1943. A guinea pig inoculation of material from a skin lesion was positive for *Coccidioides immitis* in June, 1943. Biopsies of the skin lesions were reported to show coccidioidal granuloma in February and May, 1943.

Skin tests with coccidioidin 1:1,000 and 1:100 were negative in March and October 1943. In December 1943, complement fixation tests showed a two plus reaction in the dilution of 1:64. The precipitin test was negative.

During 1943 the patient had only occasional episodes of low grade fever. In February, 1943, new lesions appeared on the sternum and perineum, and these, as well as active lesions on the nose, forehead, and left lower mandible were treated with surgical cautery, with temporary benefit.

From May, 1943 to January, 1944, the patient received several courses of saturated solution of potassium iodide in doses which ranged between 3.0 and 8.0 cc. administered three times daily. Although some healing of the lesions occurred during this therapy, they invariably relapsed when the iodide was discontinued. Moreover, the iodide therapy was accompanied by a transient enlargement of the thyroid.

On September 11, 1944, the thyroid was noted to be normal to palpation, the skin lesions were quiescent, and the patient was given a medical discharge from the Army.

The patient returned to his home in Minnesota, but was unable to return to work. A note in his record, dated September 18, 1944, states that there was no active evidence of disease.

The patient was admitted to the Minneapolis Veterans Administration Hospital in January, 1945, at which time it was noted that he felt fairly well but was still very weak. He had been taking potassium iodide irregularly and had noted that occasionally his thyroid would "swell up." Except for his appearance of malnutrition, physical examination revealed no evidence of active disease.

Roentgenograms at this time demonstrated old well-defined infiltrates in the right apex, right fourth anterior interspace,and left base, as well as a diffuse soft lesion in the left anterior second interspace (figure 2A). He was discharged after this examination.

A letter dated February 25, 1945, from his physician states that the patient had been unable to work because of marked fatigue. An active granulomatous ulcerating lesion had appeared on his nose for which he was taking potassium iodide, 5.0 cc. daily, without effect.

Roentgenograms in May, 1945 showed some increase in lesions in the right and left subapical regions. The urinalysis was negative and the hemogram was normal. One sputum examination was negative for *Coccidioides immitis*. The basal metabolism rate was plus 27. Serologic tests for syphilis were negative.

By July, 1946 his nasal lesion was noted to have increased in size, but the chest roentgenographic findings showed no change. There had been no other change during the previous year.

The patient was admitted to the Minneapolis Veterans Administration Hospital for the last time on December 11, 1947. During the preceding six months he had lost an additional 15 pounds of weight, had become weak to the point of prostration, and had developed a marked anorexia, as well as a cough productive of small amounts of sputum. In September, 1947 he had first noticed a sore throat which now made it difficult for him to swallow. He was taking potassium iodide, 3.5 cc. daily and stated that he had tried a few times during the preceding year to discontinue this medication, but that each time he did so the nasal lesions became worse.

FIG. 1. A coccidioides granuloma on forehead, nose and lip (photographed December, 1947).

On physical examination the temperature was 99.4° F., the pulse 104 per minute, and the respirations 18 per minute. The blood pressure was 90/60 mm. of mercury. It was noted that he was a poorly nourished, chronically and acutely ill, cachectic white male. There were granulomatous ulcerating skin lesions, smaller than a dime, on each side of the forehead. A large fungating, crusting, and oozing granulomatous lesion covered both nares, both alae nasae, the tip of the nose, and extended onto the upper lip (figure 1). Well healed scars were noted at the sites of the old cauterized lesions. The pharynx and tonsils were covered by ulcerating and granular lesions that completely replaced the normal mucosa. One almond-sized, freely movable nontender node was found in the right posterior cervical chain, and many smaller nodes were palpable in the neck, axillae, and groin. The left lobe of the prostate was enlarged, firm, and tender, and the left seminal vesicle was enlarged and tender. The remainder of the physical examination was negative.

The hemogram on admission was normal except for an eosinophilia of 17 per cent. The white blood cell count rose gradually to 18,300 cells per cu.mm. with a predominant polymorpho nuclear leukocytosis.

Urinalysis on admission revealed a four plus albumin reaction. The microscopic field was "solid" with white blood cells, and occasional red blood cells.

Roentgenograms of the long bones and skull were all normal in December, 1947. An intravenous pyelogram in December was also normal. Roentgenograms of the chest on admission (figure 2B) showed, in addition to the previously described metallic densities, diffuse exudative and nodular infiltrations radiating outward from the hilum through both lung fields, characteristic of a recent bronchogenic dissemination of tuberculosis or of a widespread mycotic infection. In January, 1948, roentgenograms showed clearly, for the first time, evidence of bilateral cavitation chiefly involving the upper lobes. On February 7, 1948, in addition (figure 2C), a diffuse miliary dissemination was seen throughout both lung fields.

Results of serologic and bacteriologic examinations pertaining to coccidioidomycosis and tuberculosis are given in table 1.

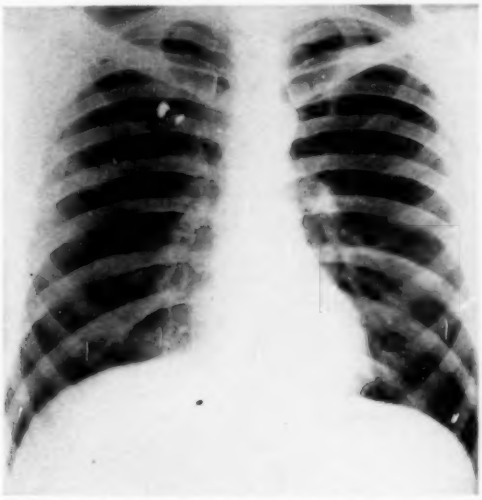


FIG. 2A. Roentgenogram in 1945, three years after onset, showing infiltration in left third interspace, right apex and right base.

Coccidioidin skin test (1:1,000 and 1:100), each performed twice, were negative. Tuberculin skin test (PPD #1 and PPD #2) were negative. Heterophile antibody and cold agglutination tests were negative.

Examination of material obtained by biopsies of the skin lesion and of the tonsil showed definite granulomatous changes with true tubercle formation. In the material obtained from the skin many typical double contoured spherules containing endospores were seen. Special staining failed to show acid-fast organisms in the skin lesion. Fewer spherules were seen in the tonsilar lesions and here many acid-fast organisms were demonstrated. Sectioned and smeared material from the sternal marrow and from the sputum showed no organisms or granulomas.

Seven relatives of the patient, with whom he had been in close association since discharge from the Army, including the patient's mother, were skin tested with coccidioidin in dilution 1:100. All were negative at twenty-four and forty-eight hours.

Cerebrospinal fluid examination on December 16, 1947, was negative. On January 15, the c.s.f. sugar was 53 mg. per 100 cc., the protein was 62 mg. per 100 cc. and microscopic examination disclosed 88 white blood cells per cu. mm., of which 90 per cent were mono-

nuclears. A first zone type of colloidal gold curve was present. Essentially similar cerebrospinal fluid findings were present on February 12.

On admission the patient was febrile with daily temperature elevations to 99.6° to 100° F. The fever subsided after one week but recurred intermittently. At the time of admission, the patient was placed on penicillin, 40,000 units every three hours, with no benefit.

Because of the marked discomfort in the pharynx, cauterization with 25 per cent trichloracetic acid was tried on two occasions without relief. By December 30, pain from the ulcerated pharynx had become so severe that the patient was able to swallow liquids only with difficulty and solids not at all. At this time he was given streptomycin 0.5 Gm. twice daily intramuscularly, which was continued until his death. Within two days the

FIG. 2B (Left). Roentgenogram at time of final hospital admission, showing extensive tuberculous and coccidioidal infiltrations.

FIG. 2C (Right). Roentgenogram three days antemortem, showing miliary coccidioidal dissemination superimposed on older processes.

patient noticed subjective throat improvement. The pharyngeal and tonsilar ulcers healed until, by February 2, the pharynx was covered by an intact mucosa.

Despite the definite healing of the pharynx, the patient's general condition gradually became worse. He complained of headache and some stiffness of the neck for the first time on January 6, and the skin lesions were noted to be larger, more swollen, and oozing considerably. On January 12, new active skin lesions appeared on the lower face and chin. The following day calciferol, 1.0 cc. (50,000 units of Vitamin D_2) orally in three doses per day was started. After two days this was changed to drisdol in propylene glycol (50,000 units of Vitamin D_2) 0.5 cc. intramuscularly twice daily. This was continued until his death. On January 14, for the first time there was definite objective neck rigidity and a positive straight leg raising test. During the ensuing three weeks, symptoms and signs of meningitis continued to wax and wane, but were never very marked.

From January 20 through January 23, the patient received 4.0 cc. of saturated solution of potassium iodide daily. On January 26, the skin granulomas were noted to be less elevated and were beginning to crust over, but within four days they had become more active, only to subside again. By February 7, the edema, erythema, and exudate had de-

creased markedly. No new skin lesions appeared after January 12, and the dermatitis never again became as active as it had been in mid-January.

On February 7, the patient rapidly became more dyspneic and weaker. The respirations were shallow, and the rate was 30 per minute. The pulse rose to 148 and the rectal temperature to 101° F. The pulse, temperature, and respiratory rates remainded elevated. Fine scattered rales and occasional wheezes were heard throughout the chest. The blood pressure remained at 84/60 mm. of mercury. A chest roentgenogram revealed the presence of a miliary dissemination. The patient became cyanotic and oxygen was given. On February 9, a pleural-pericardial friction rub was heard, and three days later a pleural

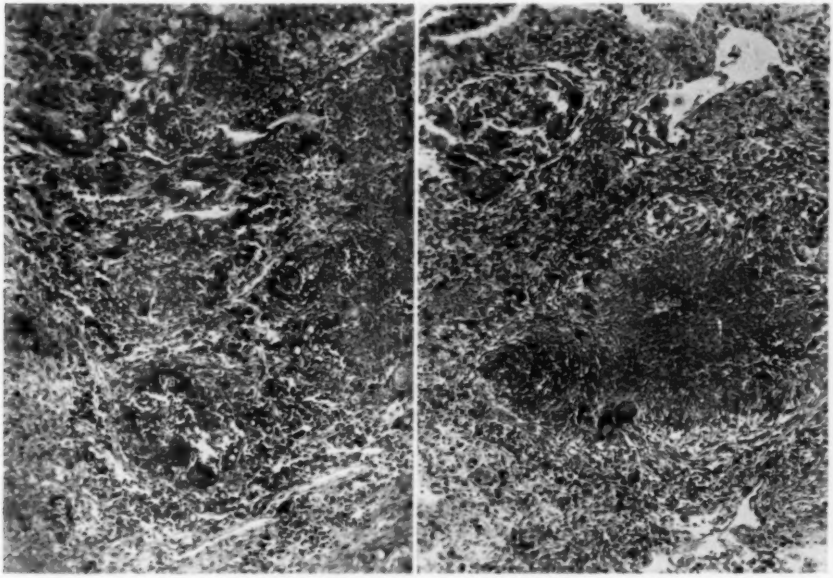


FIG. 3 (Left). A typical coccidioides granuloma from the adrenal cortex containing epithelioid cells, giant cells and coccidioides spherules.

FIG. 4 (Right). A tubercle of the lung with occasional coccidioides spherules at the periphery.

friction rub was noted. The patient became rapidly weaker and expired on February 13, 1948.

Autopsy Findings

Autopsy was performed six hours after death. The body was that of a well developed, poorly nourished white adult male weighing 110 pounds and measuring 170 cm. in length. At the external nares and covering most of the upper lip below the nose, there was a large, irregular, slightly raised granulomatous lesion extending a few millimeters into the external nares, onto both alae nasae, and the tip of the nose. It was covered by a thick, yellowish brown, firm, nodular crust and had a well defined though irregular margin, without tumescence. The surrounding skin showed no abnormalities. There were similar smaller lesions at the following sites: two on the right side of the upper lip, both one-half cm. in diameter; one on each side of the forehead, symmetrical, and measuring each one cm. in diameter; and one on the tip of the left index finger at the border of the nail.

The peritoneal surfaces were smooth and glistening. There were approximately 50 cc. of clear yellow fluid in the peritoneal cavity. The pleural cavities contained no fluid. There were numerous firm fibrous adhesions between the visceral and parietal pleural surfaces over the middle and upper lobes of the right lung and a few fibrous adhesions at the apex of the left lung. The pericardial cavity contained 30 cc. of clear yellow fluid. The pericardial surfaces were smooth and glistening. The heart showed no gross abnormalities. The lungs together weighed 2,125 Gm. There was a moderate thickening of the pleura over the upper and middle lobes of the right lung. A few pin point nodules were seen to be scatterd irregularly over the pleural surfaces of both lungs. The bronchi contained thick, pinkish fluid but in other respects showed no abnormalities. The parenchyma of the lungs was quite firm on palpation, and crepitation was reduced throughout all the lobes. On section, the entire parenchyma of both lungs had a grey solid appearance, imparted to it by closely approximated yellowish grey, punctate nodules ranging in diameter from one-half to one mm. Many of these nodules were confluent. There were several yellowish caseous-appearing nodules measuring from 3 to 5 mm. in diameter, chiefly in the apical portion of the right lung, but also in the mid-posterior portion of the left upper lobe and the upper portion of the left lower lobe. Three small cavities were seen, located respectively in each of the areas containing caseous nodules. The largest of these, measuring 2 cm. in diameter, was in the mid-posterior portion of the left upper lobe. The other two measured one cm. and one-half cm. in diameter. The cavities had fibrous walls measuring one to two mm. in thickness and contained very viscid yellow thick fluid.

The spleen weighed 225 Gm. Its parenchyma was dark purple and diffusely studded with punctate white nodules. The liver weighed 2,900 Gm. and showed no gross abnormalities. The right adrenal gland weighed 20 Gm. It was firm and slightly rubbery on palpation. The entire central and anterior portion of the gland was replaced by lobulated masses of yellowish grey, cheesy looking material. Only a small portion of the cortex remained intact. The left adrenal gland was of normal size, but had similar replacement of its central portion. In the lower portion of the ileum were numerous ulcerations which measured one-half by one cm. in diameter, with axial polarity. The margins were ragged and the bases yellowish and necrotic; the serosal coverings of these ulcerated areas were reddened and nodular. The kidneys weighed a total of 420 Gm. The parenchyma of both kidneys was diffusely studded with small white punctate nodules. In the left kidney, in the medullary portion, near one of the papillae, there was a small cystic cavity measuring one cm. in diameter. It had a smooth, thin, fibrous wall and was filled with thick greenish-yellow fluid. It did not appear to communicate with the pelvis of the kidney. The prostate was enlarged and boggy. All the ducts were dilated, giving the gland a cystic appearance, and were filled with thick yellow, creamy fluid. The seminal vesicles were moderately enlarged and bulging. They were filled with thick cheesy, greyish yellow material. The testes and epididymides showed no abnormalities.

The mediastinal, hilar, anterior cervical, and axillary lymph nodes were enlarged measuring one to two cm. in diameter. They had a rubbery firm consistency and pink homogeneous parenchyma.

On examining the brain, the leptomeninges in both cerebellopontine angles were found to be thickened, milky, and adhesive. A few small punctate, milky exudates were found along the cerebral vessels, especially in the Sylvian fissure. There were scattered punctate, granular nodules on the ventricular surfaces.

The parenchyma of the lung, on microscopic examination, was seen to be extensively disrupted by focal and diffuse granulomatous involvement, which generally had a lobular distribution and frequently gave the appearance of a granulomatous pneumonia. The interalveolar septae were largely preserved. The alveoli were filled with epithelioid cells and multinucleated giant cells, with shreds of necrotic debris, small lymphocytes, and plasma cells. Around the periphery of these areas, the alveoli were filled with macrophages, a few small lymphocytes, and contained an occasional giant cell. Numerous spherules of *Coccidioides immitis* were seen in these areas; most of these were contained in multinucleated giant cells; a few were found in macrophages.

Scattered throughout the sections of the lungs examined, in addition to the above type of granuloma, there were also seen numerous small and large masses of compact caseous necrotic material surrounded by epithelioid cells, multinucleated giant cells of the Langhans' type, and small lymphocytes. The pulmonary parenchyma was completely destroyed in these areas. Acid-fast stains revealed that many of these latter contained acid-fast bacilli in the zones of epithelioid and giant cell reaction. An occasional *Coccidioides immitis* cell was seen at the periphery of one of the small lesions of this type.

Sections of one of the cavities in the lungs revealed it to be lined by a loose fibrous capsule and to be filled with a dense mononuclear cell exudate, chiefly composed of plasma cells, but also containing macrophages and multinucleated giant cells in a loose fibrous tissue network. There were numerous coccidioidal organisms seen, most of which were in the giant cells and macrophages. The central portion of the cavity was seen to contain basophilic necrotic debris in loose clumps.

The spleen contained numerous small scattered granulomas, which contained large multinucleated giant cells, a few small lymphocytes, and plasma cells, and shreds of necrotic debris at their centers, with surrounding epithelioid cells and concentric rings of loose fibrous tissue. Occasional granulomas also contained neutrophils and eosinophils. Spherules of *Coccidioides immitis* were found in the granulomas, usually within giant cells.

The liver lobules contained a few of the same type of small granulomas, with organisms of *Coccidioides immitis*. The portal spaces were diffusely infiltrated by lymphocytes, plasma cells and a few eosinophils, and contained an occasional multinucleated giant cell.

The entire central portion of the right adrenal gland was replaced by large masses of caseous necrotic material, with only a thin rim of intact cortical cells. This material was surrounded by a broad zone of cellular reaction composed of masses of polymorphonuclear leukocytes, some small lymphocytes and plasma cells, and scattered eosinophils. The neutrophils lay in dense pockets resembling small abscesses. There were a great number of coccidioidal organisms in both the caseous area and the surrounding reaction zone. Some of these were filled with or were discharging endospores which lay in proximity to small abscesses. Most of the organisms in the cellular zone were within giant cells. The left adrenal gland was similarly involved.

The ulcers in the ileum extended to or through the muscularis mucosa, had necrotic bases and undermined edges. Under the necrotic base was found a broad zone densely infiltrated by small lymphocytes and plasma cells and containing scattered tubercles with caseous necrotic centers. No coccidioidal organisms were seen but special staining revealed the presence of numerous acid-fast bacilli.

The small cavity in the left kidney contained clumps of slightly basophilic necrotic debris, many neutrophils, and a few mononuclear leukocytes. Spherules of *Coccidioides* lay chiefly in giant multinucleated or large mononucleated cells in the peripheral portions of the cavity. The wall of the cavity was composed of a dense zone of polymorphonuclear leukocytes. Scattered throughout the remainder of the cortex and medulla of both kidneys were numerous small granulomas resembling those in the spleen, but containing a greater number of eosinophils and spherules.

The ducts and acini of the prostate contained numerous abscesses filled with neutrophils and spherules. Many of the spherules contained endospores. The abscesses were surrounded by a zone of chronic granulation tissue, containing chiefly plasma cells, some lymphocytes, eosinophils and occasional spherules.

The seminal vesicles were distended with caseous necrotic material and their epithelial lining was largely destroyed. The necrotic masses were surrounded by zones of epithelioid cells and lymphocytes extending into the surrounding stroma. No organisms of *Coccidioides* were seen, but numerous acid-fast bacilli were demonstrable with appropriate staining. The distal portions of the ducti deferens were similarly involved, but the epididymis was free of involvement.

The thyroid gland contained a few small coccidioidal granulomas located within the interlobular connective tissue, which was partially hyalinized. The lobules were free of cellular infiltrate.

Although some of the hilar nodes were uninvolved, many were filled with coccidioidal granulomas around which were dense bands of partially hyalinized connective tissue.

The lesion on the tip of the left index finger was a dense subepidermal inflammatory reaction composed of plasma cells, lymphocytes, eosinophils, and multinucleated giant cells, many of which contained *Coccidioides* spherules. There was one large subepidermal vesicle containing acidophilic fluid, many degenerating neutrophils, and a few spherules.

The leptomeninges were diffusely thickened and chronically inflamed, with numerous granulomas containing coccidoidal organisms. The same type of lesions were found in the

TABLE 1

Mycology and bacteriology of material obtained before and after death

	SPUTUM				PROSTATE			URINE				SPINAL FLUID			
	Tuberculosis smear (1)	Tuberculosis Culture	Tuberculosis Guinea pig	Coccioides Culture (2)	Tuberculosis smear	Tuberculosis Culture	Coccioides culture	Tuberculosis smear	Tuberculosis Culture	Tuberculosis Guinea pig	Coccioides culture	Tuberculosis smear	Tuberculosis Culture	Tuberculosis Guinea pig	Coccioides culture
12/15/47				+											
12/19/47	IV	+		0											
12/21/47	I														
12/22/47				0				+			0				
12/23/47		+	+	0							+				
12/26/47								+	+						
1/ 6/48								0	+		+ (4)				
1/ 7/48	VI	+		+				0	+						
1/12/48	II	+						0	+	+					
1/15/48												0	0		0
1/19/48	I			+											
1/22/48								0	0						
1/28/48	0														
2/11/48	0	+			0	0					+	0	0	0	0
2/12/48							+								+ (5)
2/13/48							+ (3)								

(1) All direct smears reported in Gaffky units.

(2) Unless otherwise indicated, all "positive" coccioides culture reports are based only upon gross examination of Sabouraud's medium culture.

(3) In addition to this "positive" culture obtained at autopsy, typical "positive" cultures were also reported from right lung, left lung, and kidney abscess. Only the last was confirmed by guinea pig inoculation.

(4) "Positive" culture, guinea pig negative.

(5) "Positive" culture, guinea pig positive.

choroid plexus and in the ependyma. Scattered granulomas containing a few organisms were also found in the white matter of the cerebral hemispheres and the pons. Acid-fast stains failed to disclose the presence of tubercle bacilli in the brain or meninges.

Acid-fast bacilli, resembling the tubercle bacillus were found only in the lungs, gastrointestinal tract, and seminal vesicles. Despite numerous attempts to demonstrate them, acid-fast organisms were not seen in any other organ.

Mycology and Bacteriology

The methods of identification of the *Coccidioides immitis* in this case were similar to those described by Dickson (2), and by Smith (8). Cultures obtained antemortem and postmortem, and their results, are shown in table 1. All of

these cultures were grown on Sabouraud's agar at room temperature. Positive cultures had an abundant white cottony growth in four to five days. From five to seven days later the growth had acquired a more fluffy appearance. Some of the growth was removed at this time from one representative culture (kidney abscess) and suspended in saline. On microscopic examination numerous chlamydospores were seen. This suspension was then injected intraperitoneally into a male guinea pig. After three weeks the guinea pig was given an intradermal injection of coccidioidin, 1:1,000. The test was read in thirty-six hours and was positive. The animal was then sacrificed. Typical spherules were recovered from an abscess of a testicle on direct smear. This process was repeated on cultures of the urine and cerebrospinal fluid, with positive results in the latter. Both the testicles and spleen of the test animal were found to contain granulomas filled with organisms of *Coccidioides immitis* on histologic examination.[4]

A record of the cultures for *M. tuberculosis* is also presented in table 1. Material for culture was concentrated and poured onto a slant of Petragagni's medium, then incubated at 37.5° C. for three to eight weeks. Positive cultures had yellow, flaky, rough colonies which were as a rule identified on macroscopic examination. Questionably positive cultures were further investigated by examination of a direct smear of the cultures. The guinea pigs were inoculated subcutaneously in the abdomen and intramuscularly in the groin with spinal fluid and sputum concentrates. After two months, the animals were sacrificed and examined for the presence of gross miliary tubercles in the spleen and liver. Using this method, only the sputum was found to contain *M. tuberculosis*.

A culture on Sabouraud's medium, obtained antemortem from the skin lesions, was sent to Dr. Robert M. Allen of the University of Nebraska College of Medicine. He injected the culture into mice and found coccidioidal spherules on histologic examination.

DISCUSSION

Histologic evidence of coccidioidal infection in this case was found in the lungs, liver, spleen, adrenals, kidneys, prostate, thyroid, meninges, brain, skin, hilar and mediastinal lymph nodes, and tonsils. There were two types of histologic response to the *Coccidioides* invasion. The first type, a granuloma composed of multinucleated giant cells surrounded by a loose net of epithelioid cells was seen throughout the organs involved. The second type, a typical suppurative lesion, was seen chiefly in the prostate and kidney. A gradation between these two types of tissue reaction was seen in the adrenals.

The granulomas in which *Coccidioides immitis* predominated differed from the tuberculous granulomas in their lack of caseation and less compact structure of the epithelioid cells (figure 3). The giant cells of the coccidioides granulomas did not show the regular peripheral crescentic placing of the nuclei as found in

[4] It may be noted that, at least in this laboratory, the testicle of the male guinea pig proved the most frequent site of growth of the coccidioidal organism after injection of culture suspensions. No growth was found after injection of culture suspensions into female guinea pigs.

the Langhans' giant cell of tuberculosis. Most of the cavities found in coccidioidal lesions were areas of liquefaction in a granuloma containing an unusual number of neutrophils. In contrast, the areas in which tubercle bacilli were found were typical caseous tubercles with Langhan's giant cells and epithelioid cells (figure 4).

This case was typical of disseminated coccidioidomycosis, in that although the prostate was involved and culture of the urine was positive, no involvement of the renal pelves or ureters was encountered. Similarly, no involvement of the bowel with *Coccidioides* was noted, although many typical tuberculous ulcers were seen.

Apparently, in this case the tuberculosis and coccidioidomycosis were coincidental. The tuberculosis was found chiefly in the lung and had disseminated only to the tonsil, bowel, and seminal vesicles. Except for the last, there was no involvement which suggested hematogenous dissemination. The coccidioidomycosis, however, had disseminated widely and was probably the cause of death.

Of special interest is the fact that, although the coccidioidin skin test was negative from the first observation in 1942, the tuberculin skin test was positive as late as May, 1943. This would indicate that, at the time the coccidioidomycosis became disseminated, at least some of the patient's immune responses were functioning normally and that his anergy to coccidioidin was an isolated phenomenon. Terminally, as both diseases became generalized, the patient also lost his ability to react to tuberculin.

Although epidemiologists have failed to demonstrate the contagiousness of human coccidioidomycosis, Rosenthal and Routien (7) have recently produced pulmonary coccidioidomycosis in guinea pigs by the intratracheal inoculation of sputum, pus, and other material from cases of human coccidioidomycosis. In view of these writers' conclusions that sputum and other material "can be infective through the respiratory route," it is interesting to note that 7 individuals who had lived in close association with our patient over a period of thirty-nine months demonstrated neither positive skin tests nor any other evidence of coccidioidomycosis.

The present case illustrates again the lack of any curative therapeutic attack upon disseminated coccidioidomycosis. During the course of the disease, sulfonamides, penicillin, streptomycin, fever therapy, local cautery, potassium iodide, and massive doses of vitamin D_2 were all tried. The first three were of no value. The fever therapy may have been of some temporary benefit, as was the local cautery. Although numerous other cases have been placed on potassium iodide with no benefit, this medication apparently produced a definite retarding effect on the progress of our patient's coccidioidomycosis. The effect of the iodide on the tuberculous processes is more obscure. Although potassium iodide was begun in May, 1943 and continued intermittently until December, 1947, acid-fast bacilli were not noted in the patient's sputum until the latter date. At this time iodide therapy was discontinued. Four cc. of potassium iodide daily were administered from January 20 through January 23, 1948, with no

obvious change in the course of the tuberculosis. It is not possible on the basis of this evidence to draw any definite conclusion as regards the influence of potassium iodide on tuberculosis.

Neither can conclusions be drawn in regard to the use of massive doses of vitamin D_2 in coccidioidomycosis of the skin. However, the coccidioidal lesions cleared sufficiently during the period of administration of calciferol to warrant further clinical trial of the drug.

Perhaps the most interesting observation was that of the differential effect of streptomycin, which it is believed definitely aided the healing of the patient's pharyngeal tuberculosis, while his coccidioidomycosis grew worse.

SUMMARY AND CONCLUSION

A case of disseminated coccidioidomycosis which lasted for six years and was complicated by active tuberculosis is presented. The two diseases seemed to occur coincidentally, the individual coccidioidal granulomas being devoid of acid-fast bacilli, and the tubercles containing only a few *Coccidioides* spherules at the periphery. The coccidioidomycosis terminated in a miliary dissemination throughout the body, including the brain and prostate, as well as the more usual sites, but did not involve the bowel or renal pelves. The tuberculosis presented a definite "secondary" or "adult" type of distribution in the lungs, pharynx, and bowel, as well as a hematogenous dissemination to the seminal vesicles.

Streptomycin was of definite value in controlling the tuberculosis but did not affect the coccidioidomycosis, whereas potassium iodide and vitamin D_2 in massive doses seemed to decrease some manifestations of the coccidioidomycosis. It is suggested that the latter drugs be given further trial in cutaneous coccidioidomycosis.

SUMARIO Y CONCLUSIONES

Coccidioidomicosis Difusa Coexistente con Tuberculosis

El caso presentado es de coccidioidomicosis difusa de seis años de duración, complicado por tuberculosis activa. Las dos enfermedades aparentemente coincidieron en su aparición, hallándose los distintos granulomas coccidioideos desprovistos de bacilos ácidorresistentes y conteniendo los tubérculos apenas algunas esférulas de *Coccidioides* en la periferia. La coccidioidomicosis terminó en difusión miliar por todo el cuerpo, incluso el cerebro y la próstata así como los sitios más habituales, pero sin afectar el intestino o las pelvis renales. La tuberculosis mostró una forma de distribución "secundaria" o "adulta" bien definida en los pulmones, faringe e intestino, así como difusión hematógena a las vesículas seminales.

La estreptomicina resultó de valor decidido en lo relativo a cohibir la tuberculosis, pero no afectó la coccidioidomicosis, en tanto que el yoduro de potasio y la vitamina D_2 a dosis masivas parecieron atenuar algunas manifestaciones de la última. La última droga podría muy bien ser objeto de nuevas pruebas en la coccidioidomicosis cutánea.

Acknowledgment

Dr. William B. Tucker, Chief of the Tuberculosis Service, Minneapolis Veterans Administration Hospital, and Dr. C. E. Smith of the Department of Public Health and Preventive Medicine, Stanford University Medical School, have given invaluable aid in the preparation of this paper. To the members of the staff of the 73rd Evacuation Hospital, the Hoff General Hospital, and the McCloskey General Hospital, we are indebted for the excellent study they made of the early course of this case.

REFERENCES

(1) Cherry, C. B., and Bartlett, A. G.: The diagnosis of acute coccidioides immitis infection, Bull. U. S. Army M. Dept., 1946, *5*, 190.

(2) Dickson, E. C.: Coccidioides infection, Arch. Int. Med., 1937, *59*, 1029.

(3) Forbus, W. D., and Bestebreurtje, A. M.: Coccidioidomycosis, Mil. Surgeon, 1946, *99*, 653.

(4) McDougall, T. G., and Kleiman, A. H.: Prostatitis due to coccidioides immitis, J. Urol., 1943, *49*, 472.

(5) Rakofsky, M., and Knickerbocker, T. W.: Roentgenologic manifestations of primary pulmonary coccidioidomycosis, Am. J. Roentgenol., 1946, *56*, 141.

(6) Rifkin, H., Feldman, D. J., Hawes, L. E., and McGrath, L. E.: Coexisting tuberculosis and coccidioidomycosis, Arch. Int. Med., 1947, *79*, 381.

(7) Rosenthal, S. R., and Routien, J. B.: Contagiousness of coccidioidomycosis, Arch. Int. Med., 1947, *80*, 342.

(8) Smith, C. E.: Coccidioidomycosis, M. Clin. North America, 1943, *27*, 790.

(9) Smith, C. E.: Parallelism of coccidioidal and tuberculous infections, Radiology, 1942, *38*, 643.

(10) Study, R. S., and Morgenstern, P.: Coexisting pulmonary coccidioidomycosis and tuberculosis, New England J.Med., 1948, *238*, 837.

(11) Sweigert, C. F., Turner, J. W., and Gillespie, J. B.: Clinical and roentgenologic aspects of coccidioidomycosis, Am. J. M. Sc., 1946, *212*, 652.

VIABILITY OF TUBERCLE BACILLI IN EMBALMED HUMAN LUNG TISSUE[1]

GORDON M. MEADE AND WILLIAM STEENKEN, JR.

(Received for publication April 26, 1948)

INTRODUCTION

In the course of a study of tuberculin conversions occurring among medical students at the University of Rochester School of Medicine, Rochester, New York, it was found that the majority of conversions which occurred during the four years of the medical curriculum occurred during the first and second academic years. By far the majority occurred during the second year and it was concluded that these were due to contact with tuberculosis autopsy material (1). It was also noted, however, that a certain number of conversions took place during the first academic year when there was no known contact with cases of active pulmonary tuberculosis or with tuberculosis autopsy tissues. It seemed of interest, therefore, to investigate the possibility of infection of students from the tissues of cadavers used for dissection purposes in courses in anatomy. Dissection cadavers are always embalmed and it has been generally assumed that embalming suffices to kill any tubercle bacilli present. However, a search of the literature revealed no study covering this particular point. With this in mind it was decided to investigate the question of the viability of tubercle bacilli in embalmed cadavers which had known pulmonary tuberculosis. This paper reports the results of such a study.

METHOD

A letter outlining the problem and the reason for the investigation was sent to approximately eighty departments of anatomy in schools of medicine in the United States and Canada. A request was made that specimens of lungs from cadavers used for dissection purposes which were known, or suspected, to have had pulmonary tuberculosis during life be removed under as sterile conditions as possible and sent to us for study. It was requested that specimens be taken from previously undisturbed portions of the diseased lung; that they be removed under as aseptic conditions as possible, using clean sterilized instruments. It was also requested that specimens should be large enough that untouched areas could be dissected out upon receipt. Specimens were to be mailed in clean, preferably sterile, jars or other glass containers. On each specimen submitted, certain pertinent information was requested, to include the date of death, the stated cause of death, the date of embalming, the type and strength of embalming fluid used, the method of storage after embalming and the date the specimen was taken from the cadaver.

Splendid cooperation was experienced and, in all, specimens were received from a total of sixteen different sources.

Upon receipt the specimens were placed in refrigeration until examination and use of them could be made. Each specimen was separately examined and dissected with clean, sterilized instruments for gross evidence of tuberculosis. A number of specimens were discarded because in the portion submitted to us no gross evidence of tuberculosis could be found. From those specimens showing gross evidence of tuberculosis pieces of

[1] From the Trudeau Sanatorium and Edward L. Trudeau Foundation.

involved tissue were removed, crushed and smeared, stained by Ziehl-Neelsen method, and examined at once for presence of tubercle bacilli. No tissue was used in the experiment, with one exception which did not show tubercle bacilli on direct smear. Portions of suspected tissue were also taken for histological study. After direct smear examination, portions of tissue from the same area of the specimen were triturated in physiological saline and injected into guinea pigs previously found negative to 5 per cent Old Tuberculin. Two guinea pigs were used for each specimen. Following the inoculation, tuberculin tests were performed at the end of two and three months. The usual criterion of at least 5 mm. of induration with or without erythema was used in reading the tuberculin tests. Three months after inoculation all surviving guinea pigs were sacrificed and examined for evidence of tuberculosis.

RESULTS

Interval between death and embalming: In the 24 specimens used in the experiment, the interval between death and embalming varied from 0 to 15 days with an average interval of slightly over 4 days. During this interval the bodies were presumably refrigerated the greater part of the time.

Method of embalming: All of the bodies were embalmed by the departments of anatomy to which they were sent, but in a few instances there was primary embalming of a commercial type by an undertaker or professional embalmer followed by a second embalming after delivery to the school of medicine. The method of the primary embalming is unknown.

The embalming methods used varied somewhat in detail as did the fluids. There was no one ingredient common to all the fluids. Phenol in concentrations of from 4.5 to 33 per cent was the most common constituent. Then followed glycerin in concentrations of 16 to 40 per cent; alcohol 10 to 45 per cent; and lastly formalin 3 to 25 per cent. The most commonly used combination contained all four substances. Some of the fluids contained, besides water, such other materials in small amounts as sodium chloride, sopronol, potassium nitrate, and arsenic trioxide. So far as could be seen in this rather limited amount of material, all types of fluid seemed to preserve the tissues equally well.

Duration of embalming: A factor which might have a definite influence on the survival of the tubercle bacilli is the length of time between death of the host and the attempt at isolation in a new, living host; in short, the interval between death of the bodies in question and the subsequent inoculation into guinea pigs. The ultimate length of life of tubercle bacilli in an unembalmed, refrigerated body is not known. The writers previously found in one body that tubercle bacilli remained viable in large numbers under such conditions for at least three months. Thus, the fate of the organisms is possibly the resultant of two factors, namely, the effect of death of the host, and of the embalming fluid. There was not sufficient variety of material to make a comparison of the various time intervals after death with each of the various fluids. However, in the 24 specimens the interval between death of the host and injection of the bacilli-laden tissues into guinea pigs varied from three months to four years. The average was twenty-two and one-half months. The body which had been embalmed for only three months contained many tubercle bacilli on direct smear of the tissue. The embalming fluid used consisted of 25 per cent glycerin, 25 per cent formaldehyde and 50 per

cent water. Four bodies had been embalmed only six months; all showed many organisms on direct smear. One had been embalmed with the 25 per cent glycerin, 25 per cent formaldehyde, 50 per cent water formula; one with arsenic trioxide, phenol, formalin, water and KNO_3; and one with a formula the composition of which was uncertain.

Identification of organisms direct from tissues: In all instances material, usually caseous, was smeared directly on glass slides, stained for acid-fast organisms in the usual manner and examined at once before guinea pig inoculation. An approximation of the number of organisms seen was made, using the terms *many, few, occasional* and *none.* Thirteen of the 24 specimens were rated as having many organisms (usually this meant that the slide showed a myriad of organisms); four were rated as showing few organisms; six were rated as showing occasional; and there was only one specimen which showed no organisms on smear. In the case of this last named specimen, however, the stated cause of death was pulmonary tuberculosis and there were both gross and histologic evidences of tuberculosis. On smear the organisms from all specimens were typical acid-fast organisms which resembled tubercle bacilli in every respect and presented no abnormalities of staining properties or morphology.

Histologic examination of tissues: The tissue taken from the specimens for histological study in every instance showed typical tuberculous reactions.

Results of tuberculin tests: All guinea pigs were tuberculin skin tested two and three months after inoculation. Tests were made with 5 per cent Old Tuberculin. Table 1 shows the results of these testings. The usual standard for a positive reading of 5 by 5 mm. of induration with or without erythema was used. A 5 to 10 mm. reaction was rated as 1+; 10 to 20 mm. as 2+. The guinea pigs from 17 of the 24 specimens were entirely negative throughout the experiment with respect to tuberculin reactions. Some of the test animals showed definitely positive reactions at the end of two months but showed a negative or doubtful reaction at the end of three months. The animals inoculated from one specimen, A-25, were notable in that both guinea pigs showed definitely positive reactions when examined at two and again at three months after injection. Both of these animals were also the only ones of the entire group which showed any abnormality on postmortem, namely, slight, firm, noncaseating enlargement of the inguinal and iliac lymph nodes with no gross evidence of tubercle formation.

It is not unjustified to speculate that the positive tuberculin reactions with negative evidence of disease were induced by the presence of large numbers of dead tubercle bacilli. The reality of the establishment of hypersensitivity in such a manner has been well established (2, 3). In the present study, the measurement of the numbers of tubercle bacilli present was very rough but possibly gave some comparative estimate. In those 7 animals which at two or three months showed a positive tuberculin reaction, the rating of number of tubercle bacilli on smear was many in 5, occasional in one, and none in one. On the other hand there were 8 specimens rated as having many organisms whose inoculated guinea pigs showed no, or questionable, evidence of a positive reaction.

Results of guinea pig inoculation: In all, 48 guinea pigs were inoculated, two

TABLE I

Data on specimens of embalmed lung tissue, arranged in order of duration of embalming

SPECIMEN NUMBER	INTERVAL DEATH TO EMBALMING	INTERVAL EMBALMING TO ANIMAL INOCULATION	EMBALMING FLUID	METHOD OF STORAGE AFTER EMBALMING AND BEFORE DISSECTION	NUMBER ORGANISMS IN SMEAR	POSTINOCULATION TUBERCULIN TEST			GUINEA PIG AUTOPSY 12 WEEKS AFTER INOCULATION
							2 months	3 months	
	days	*months*							
A-14	7	3	Formalin—25 per cent Glycerin—25 per cent H_2O—50 per cent	Refrigerated at 32°F.	Many	Pig 1..... Pig 2.....	0 0	0 0	Negative Negative
A-4	15	6	Arsenic trioxide, phenol, formalin, H_2O, KNO_3	Immersed in 4 per cent aqueous phenol at room temperature	Many	Pig 1..... Pig 2.....	Not done 0	Not done 0	Pig died 28 days after inoculation. No tuberculosis. Negative
A-6	7	6	Same as A-4	Same as A-4	None	Pig 1..... Pig 2.....	0 1+	0 Not done	Negative Died 50 days after inoculation of streptococcus pneumonia. No tuberculosis.
A-9	?	6	Same as A-4 or A-7	Same as A-4	Many	Pig 1..... Pig 2.....	0 1+	0 0	Negative Negative
A-19	5	6	Same as A-14	Same as A-14	Many	Pig 1..... Pig 2.....	1+ 0	0 0	Negative Negative
A-16	2	7	Same as A-14	Same as A-14	Many	Pig 1..... Pig 2.....	0 0	0 0	Negative Negative

A-7	10	11	Alcohol—39 per cent Formalin—3 per cent Glycerin—39 per cent Phenol—19 per cent	Same as A-4	Many	Pig 1..... Pig 2.....	1+ Not done	1+ Not done	Negative Died 21 days after inoculation of streptococcus pneumonia. No tuberculosis.
A-15	1	11	Same as A-14	Same as A-14	Occasional	Pig 1..... Pig 2.....	1+ 1+	0 ±	Negative Negative
A-5	7	12	Same as A-7	Same as A-4	Many	Pig 1..... Pig 2.....	0 0	± Not done	Negative Died 45 days after inoculation. No tuberculosis.
A-13	5	13	Same as A-14	Same as A-14	Few	Pig 1..... Pig 2.....	0 0	0 0	Negative Negative
A-18	0	14	Same as A-14	Same as A-14	Many	Pig 1..... Pig 2.....	Not done 0	Not done 0	Died accidental death 18 days after inoculation. No tuberculosis. Negative
A-17	4	16	Same as A-14	Same as A-14	Few	Pig 1..... Pig 2.....	0 0	0 Not done	Negative Died 67 days after inoculation of streptococcus pneumonia. No tuberculosis.
A-30	3	18	Alcohol—33 per cent Glycerin—33 per cent Phenol—33 per cent	Immersed in 2 per cent phenol	Occasional	Pig 1..... Pig 2.....	0 0	0 0	Negative Negative

TABLE I—*Continued*

SPECIMEN NUMBER	INTERVAL DEATH TO EMBALMING	INTERVAL EMBALMING TO ANIMAL INOCULATION	EMBALMING FLUID	METHOD OF STORAGE AFTER EMBALMING AND BEFORE DISSECTION	NUMBER ORGANISMS IN SMEAR	POSTINOCULATION TUBERCULIN TEST			GUINEA PIG AUTOPSY 12 WEEKS AFTER INOCULATION
							2 months	3 months	
	days	*months*							
A-11	5	26	95% Alcohol—33 per cent Glycerin—33 per cent Phenol—33 per cent	Immersed in 5 per cent cresylic acid solution	Many	Pig 1..... Pig 2.....	0 0	0 0	Negative Negative
A-12	4	28	Same as A-11	Same as A-11	Many	Pig 1..... Pig 2.....	2+ 1+	± ±	Negative Negative
A-10	4	29	Same as A-11	Same as A-11	Many	Pig 1..... Pig 2.....	0 ±	0 0	Negative Negative
A-1	5	36	95% Alcohol—33 per cent Formalin—9 per cent Glycerin—16 per cent NaCl—1.6 per cent Phenol—4.5 per cent Sopronol—5 per cent H_2O—31 per cent	Greased, wrapped, stored at 37°F.	Many	Pig 1..... Pig 2.....	0 0	0 0	Negative Negative
A-23	4	38	Alcohol 10 per cent Formalin Glycerin Phenol	Immersed in 2 to 3 per cent phenol	Occasional	Pig 1..... Pig 2.....	0 0	0 0	Negative Negative

A-24	2	39	Same as A-23	Same as A-23	Few	Pig 1.....	0	0	Negative
						Pig 2.....	±	0	Negative
A-29	2	40	Same as A-30	Same as A-30	Occasional	Pig 1.....	0	0	Negative
						Pig 2.....	0	0	Negative
A-28	1	42	Same as A-23	Same as A-23	Occasional	Pig 1.....	0	0	Negative
						Pig 2.....	0	0	Negative
A-25	1	45	Same as A-23	Same as A-23	Many	Pig 1.....	1+	1+	Sl. enlarged, firm, non-caseous inguinal and iliac nodes. No tuberculosis.
						Pig 2.....	1+	1+	Sl. enlarged, firm, non-caseous inguinal and iliac nodes. No tuberculosis.
A-27	1	46	Same as A-23	Same as A-23	Occasional	Pig 1.....	0	0	Negative
						Pig 2.....	0	0	Negative
A-26	3	48	Same as A-23	Same as A-23	Few	Pig 1.....	0	0	Negative
						Pig 2.....	0	0	Negative

for each of the 24 specimens. During the course of the experiment 6 animals died: one of an accident, 3 of pneumonia, and 2 of unknown cause. None of these animals showed any evidence of tuberculosis at the time of death which occurred from 18 to 67 days after inoculation. In no instance did both the animals inoculated from any one specimen die so that at least one animal inoculated from each specimen was available to complete the experiment.

No animals died of tuberculosis during the experiment. All surviving animals were sacrificed at the end of 12 weeks. No gross evidence of tuberculosis was found in any of them. In both animals of one specimen (A-25) there was slight, firm, noncaseating enlargement of the inguinal and iliac lymph nodes.

COMMENT

In view of the all too frequent infection of medical students with tuberculosis and the observation that some primary infections regularly occur during the first year of the medical curriculum (1), it seemed advisable to establish whether or not the dissection of cadavers which had pulmonary tuberculosis in life constitutes a possible source of infection. The question of viability of tubercle bacilli in embalmed tissues doesnot seem to have been dealt with in the literature on tuberculosis. The present study does not pretend to be a definitive one in that no attempt was made to establish standard embalming techniques nor minimal or maximal survival times. The study simply aims to show whether tubercle bacilli survive in the lungs of anatomy dissection cadavers under the usual techniques and practices of schools of medicine. It would appear from this study that in all probability the organisms are nonviable after three to six months with the methods in general use. No instance was encountered where the body was used for dissection sooner than three months after embalming.

Since this study was confined to the lungs of the embalmed bodies, the findings and conclusions can strictly be applied only to this organ tissue. It is conceivable that the penetration of embalming fluids to other tissues might not be as complete and that there might thus be occasional survival elsewhere. It is also possible that at times a portion of a lung or other organ might not be adequately embalmed because of blockage of embalming fluid from that area by an occluded vessel. In such an instance it is conceivable that there might be local survival of tubercle bacilli.

The development of positive tuberculin reactions in some of the animals without evidence of disease suggests the possibility that tuberculous dissection material might be a source of a tuberculin conversion because of the presence of dead tubercle bacilli if a sufficient amount of material entered the body. Such a possibility, however, seems quite remote and hardly worth consideration.

SUMMARY AND CONCLUSIONS

1. Tubercle bacillus laden lung tissue from 24 anatomy dissection cadavers obtained from schools of medicine was injected into guinea pigs.

2. Some of the injected animals developed positive tuberculin tests at the end of two or three months.

3. No guinea pigs died of tuberculosis and none showed evidence of disease at autopsy three months after inoculation.

4. It is concluded that under the conditions ordinarily prevailing tubercle bacilli are not viable in lungs of embalmed Medical School dissection cadavers at the time of dissection.

SUMARIO Y CONCLUSIONES

Viabilidad de los Bacilos Tuberculosos en el Tejido Pulmonar Humano Embalsamado

1. Inyectóse en cobayos tejido pulmonar cargado con bacilos tuberculosos, obtenido de 24 cadáveres utilizados para disección anatómica en facultades de medicina.

2. Algunos de los animales inyectados manifestaron reacciones positivas a la tuberculina al cabo de dos o tres meses.

3. Ningún cobayo murió de tuberculosis ni ninguno mostró signos de enfermedad en la autopsia a los tres meses de la inoculación.

4. Dedúcese que, en las condiciones que reinan habitualmente, los bacilos tuberculosos no son viables en los pulmones de los cadáveres embalsamados para disección al hacerse la última.

REFERENCES

(1) Meade, G. M.: The prevention of primary tuberculous infections in medical students, Am. Rev. Tuberc., 1948, *58*, 675.

(2) Petroff, S. A., and Stewart, F. W.: Concerning allergic reactions obtained in animals sensitized with killed tubercle bacilli, J. Immunol., 1925, *10*, 677.

(3) Rich, A. R.: The Pathogenesis of Tuberculosis, Charles C Thomas, Springfield, Ill., 1944, p. 350.

ACQUIRED RESISTANCE OF M. TUBERCULOSIS TO STREPTOMYCIN IN VITRO AND ITS BEHAVIOR IN EXPERIMENTAL INFECTIONS[1, 2]

E. W. EMMART, W. T. McCLOSKY, M. I. SMITH AND J. E. LIEBERMAN[3]

(Received for publication February 20, 1948)

The development of resistance *in vivo* of the tubercle bacillus to streptomycin in man in the course of treatment was reported by Youmans and associates (1). More recently Williston and Youmans (2) have also shown that the degree of acquired resistance *in vitro* varied with different strains. Using a culture medium consisting of a synthetic broth with plasma to which streptomycin was added, 4 out of 18 strains failed to acquire resistance, one strain developed a twofold resistance, 3 only a fourfold resistance while 9 strains showed a thousandfold resistance. These results are in accord with those of Karlson, Feldman and Hinshaw (3) who demonstrated that resistance of strains from treated tuberculous patients varied from .08 to 2,000 γ per cc. and that the acquired resistance was not lost upon passage through guinea pigs and subsequently maintained for many weeks on solid culture media.

The present report presents data on the acquisition of resistance *in vitro* by the virulent slow growing human strain A27; the difference in growth rate of the resistant and control strains; the relative degree of invasiveness of these strains in the chorio-allantois of the chick embryo and in the guinea pig; and the efficacy of streptomycin therapy in experimental infections in guinea pigs inoculated with the resistant strain.

RESULTS

Acquired resistance in vitro and the growth rate of the resistant strain: The human strain A27 used in these experiments had initially a sensitivity of 1.0 to 1.5 γ per cc. (4). After a series of seventeen successive passages in Kirchner's medium with increasing concentrations of streptomycin, beginning with an initial concentration of 0.5 γ per cc., the strain grew well in a concentration of 5.0 γ per cc.

After the strain had developed a tolerance to 4.0 γ per cc. streptomycin, it was transferred to the control medium for two generations totaling approximately 70 days and then its growth rate was compared with that of the parent strain. Three experiments were set up, each in triplicate. The average growth of the 9 flasks for both the control and resistant strains was determined at intervals of 5 days and recorded as previously described (5).

At the end of 45 days, when the experiment was terminated, the growth of the control strain covered the whole surface of the culture medium and was rated 4.0,

[1] From the Experimental Biology and Medicine Institute, National Institute of Health, Bethesda, Maryland.

[2] Presented at the Antibiotics Study Section Seminar, National Institute of Health, October 1, 1947.

[3] Biostatistician, Division of Public Health Methods, U. S. Public Health Service.

while the growth of the streptomycin-resistant strain averaged 2.2. After the initial lag period of approximately 12 days, the growth curve of the control strain was found to be consistently higher than that of the resistant strain (figure 1) and at the end of the experimental period of 45 days the growth of the resistant strain was about 55 per cent of that of the control strain. Since the rate of growth of the two strains within a given period of time may be the basis for testing tuberculostatic action *in vitro*, it was logical to assume that the comparison of the growth curves over the entire experimental period would furnish more information than the single comparison of growth at the termination of the experiment. In this

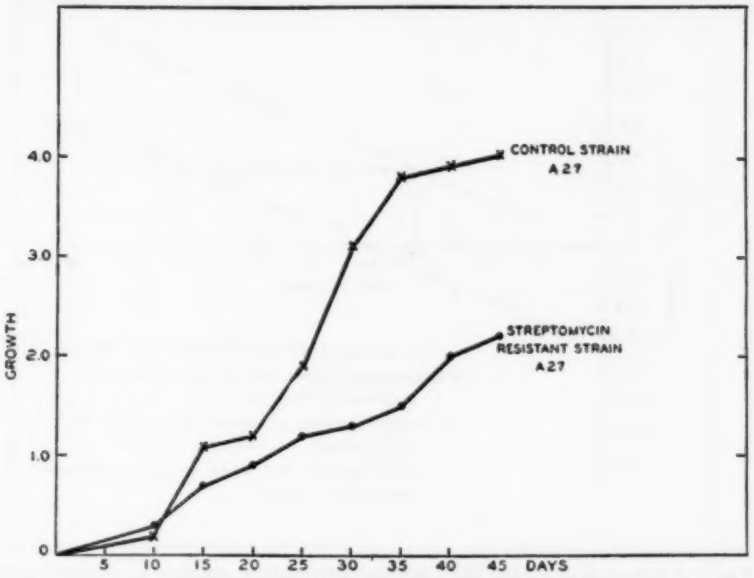


FIG. 1. Difference in growth rate of streptomycin-resistant strain (A27) as compared with the control strain in Kirchner's medium. The points represent the average growth of 9 flasks at successive intervals of time.

respect the problem is similar to that of a bio-assay (6, 7) in which the effect of a biological product is determined over a range of concentrations and not at just one concentration.

In adopting a bio-assay technique it was necessary to make the assumption that the logarithm of the growth measurement has a linear relation to the time. Tests of this assumption have been made on the data under consideration and no significant departure from linearity has been found. In the statistical analysis of the difference in the growth of the control and resistant strains, the mean growth readings for each strain were converted into logarithms and straight lines fitted to the points by the method of least squares (8). In figure 2 are represented the recti-

fied growth curves shown in figure 1. The equations of these rectified curves as determined by least squares are—

$$y_1 = 0.0054 + 0.015\, t_1$$

and

$$y_2 = 0.3305 + 0.016\, t_2$$

where y_1 and t_1 are respectively the log growth and the time for the control strain, and y_2 and t_2 stand for the same variables in the resistant strain.

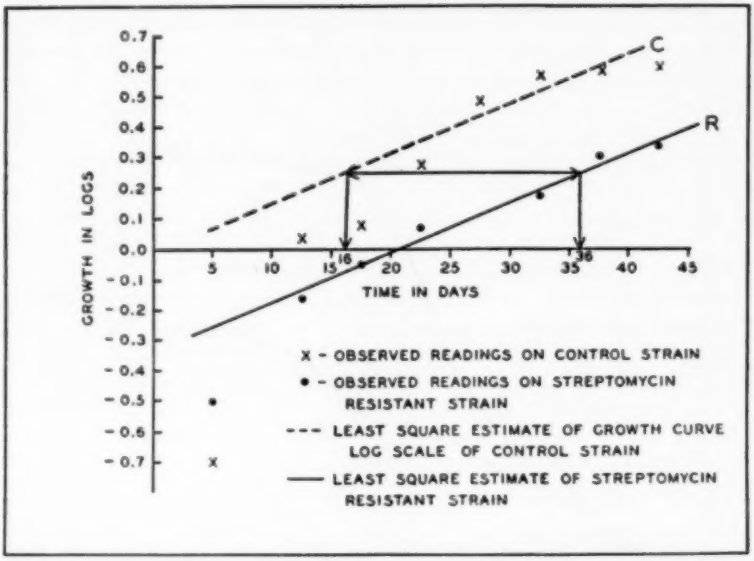


FIG. 2. Log growth of control and streptomycin-resistant strains with adjusted curves by the method of least squares. The difference of 36 and 16, or 20, days represents the difference in time required for the resistant strain to reach the same amount of growth as the control strain.

These rectified curves are also plotted in figure 3 and 4 on semi-log paper. In these figures are shown the curves representing a multiple of the standard error of estimation of the average growth at each point along the curve. In figure 3 this is represented for the control strain by the 95 per cent confidence band.[4] The minimum error of estimation occurs near the growth of 1.8 which in log units is near 0.25 (figure 2). In figure 4 the confidence band for the resistant strain is shown.

[4] Since the growth rate was greater in the control strain, the observable deviations from the mean were greater. This is represented by the wider spread of the confidence band (figure 3) as compared with the confidence band of the resistant strain (figure 4). This also explains the deviation of some of the points from the straight line C in figure 2.

Using the log growth of 0.25 as the estimated growth at the point of approximate minimum error, the time when the control and resistant strains reached this growth could then be determined. In figure 2 this point on the curve of the control strain corresponds to 16 days. The same amount of growth on the curve of the resistant strain corresponds to 36 days. The difference of 36 minus 16, or 20 days, is the measure of retardation of growth of the resistant strain.

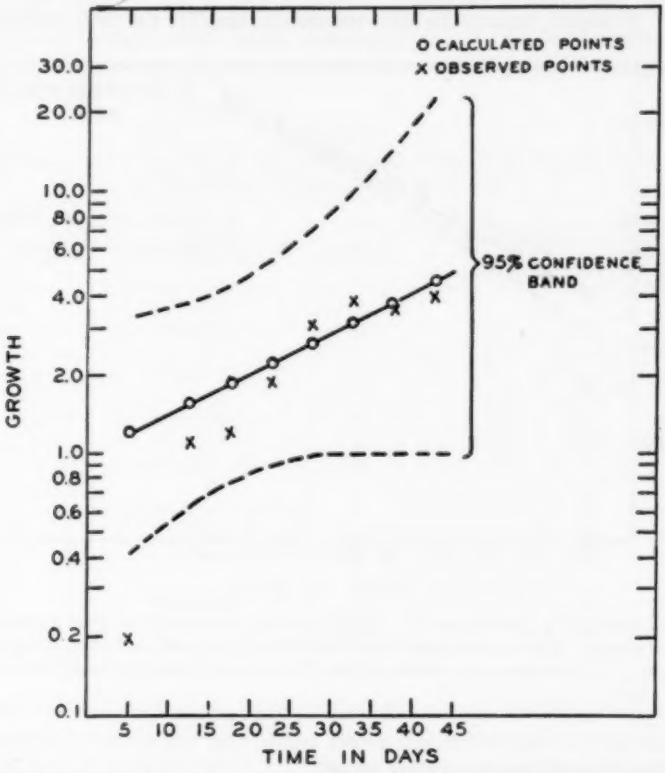


FIG. 3. Rectified growth curve of the control strain and the 95 per cent confidence band. The minimum error of estimation is the point on the growth band which most closely approaches the confidence band.

Invasiveness of the streptomycin-resistant and control strains in the chorio-allantoic membrane of the chick embryo: In order to determine the relative degree of invasiveness of the 2 strains in the chorio-allantoic membrane of the chick embryo, 7 experiments were carried out in which the resistant and the control strains were each inoculated on the membranes of the 8 day old chick embryo. The technique of preparation and mode of inoculation were the same as previously described (9, 5) with 1.0 mg. of bacilli in each 0.2 cc. of inoculum. In these experiments the

resistant strain was obtained from the fifth to the sixteenth passage in 0.5, 1.0 and 4.0 γ per cc. of streptomycin respectively. The membranes were harvested 6 days after inoculation and the inoculated areas fixed, excised, and the size and number of tubercles counted. Table 1 gives the average tubercles per membrane for the experimental and control groups for the 7 experiments. From the data obtained during the brief 6 day experimental period, it was not possible to demonstrate a consistent difference in the degree of invasiveness of the resistant and control strains. However, some of the same suspensions used for the chick embryo tests

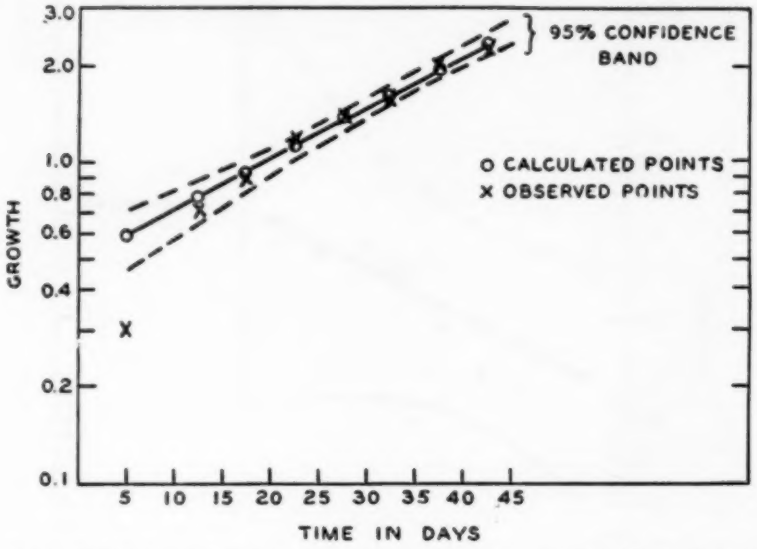


FIG. 4. Rectified growth curve of the slowly growing resistant strain with the 95 per cent confidence band. The curves representing the limits of the confidence band are based upon a multiple of the standard error of estimation of the average growth at each point along the growth curve.

were also used for inoculation into guinea pigs so that the course of the infection might be observed during a longer period.

Pathogenicity of the resistant and control strains after inoculation into guinea pigs: To determine the degree of invasiveness of the streptomycin-resistant strain as compared with the control in guinea pigs, 4 experiments were carried out with some of the same bacterial suspensions as were used in the preceding experiments. In experiment 1, inoculations were made from suspensions after the fifth passage; in experiment 2 after the seventh passage in 0.5 γ per cc. streptomycin; in experiment 3 from suspensions of the twelfth transfer in 3.5 γ per cc. streptomycin; and in experiment 4 from suspensions of cultures of the sixteenth transfer in 4.0 γ per cc. streptomycin. At the time of the twelfth passage the strain in strepto-

mycin had developed a two-to threefold resistance. The dosages injected intraperitoneally were as follows: experiments 1 and 2, 1 mg.; experiment 3, 0.25 mg.; and experiment 4, 0.5 mg.

Experiments 1, 2 and 3 were terminated 42 and 43 days after inoculation while experiment 4 was terminated 105 and 106 days after inoculation. The degree of invasiveness of the strain was based upon the extent of tuberculous involvement of the liver, spleen, omentum, peritoneum and kidneys and lungs as previously described (10). In experiment 1, the difference in the tuberculosis index between the control and experimental groups was negligible. In the last 3 experiments, the tuberculosis index was slightly less in the experimental groups than in the control groups (table 2).

TABLE 1

Invasiveness of the streptomycin-resistant (R) and control (C) strains of A27 in the chorio-allantoic membrane of the chick embryo

EXPERIMENT NUMBER	STRAIN OF TUBERCLE BACILLI*	NUMBER OF PASSAGES AND AMOUNTS OF STREPTOMYCIN	NUMBER OF EMBRYOS SURVIVING	AVERAGE NUMBER OF TUBERCLES PER MEMBRANE			TOTAL AVERAGE NUMBER OF TUBERCLES PER MEMBRANE
				Large	Medium	Small	
		γ per cc.					
257	R	Fifth—0.5	16	0.7	3.1	14.0	17
	C		16	1.2	3.8	13.1	17
264	R	Sixth—0.5	8	0.2	1.1	6.7	8
	C		10	0.5	1.5	12.2	14
268	R	Seventh—0.5	10	1.0	3.3	5.6	10
	C		12	0.7	1.0	6.3	8
282	R	Tenth—1.0	13	0.0	0.2	17.0	17
	C		13	0.1	0.2	17.0	18
300	R	Fourteenth—4.0	22	0.2	0.3	0.9	1.3
	C		20	0.2	0.1	0.8	1.0
301	R	Fifteenth—4.0	23	0.2	0.3	4.0	4.6
	C		21	0.4	0.9	9.6	10.9
302	R	Sixteenth—4.0	25	0.2	0.6	1.4	2.1
	C		18	0.2	0.5	1.2	1.4

* R = resistant; C = control.

All the inoculated guinea pigs were weighed once a week and the average weight for each group calculated and plotted. Inspection of figures 5 and 6 will show that in each of these experiments the weight curves of the guinea pigs inoculated with the resistant strain were consistently higher than those inoculated with the control strain.

The differences in the weight curves and the tuberculosis index of the guinea pigs inoculated with the streptomycin-resistant strain and the control strain may possibly be explained by the difference in growth rates of the 2 strains as shown in the *in vitro* experiments or by a possible reduction in virulence or both. Yegian, Budd and Middlebrook (11) have demonstrated a slower growth rate of *M. ranae* with acquired resistance to certain sulfonamides.

Streptomycin therapy in guinea pigs inoculated with streptomycin-resistant and

control strains: In order to determine the effect of streptomycin therapy in guinea pigs inoculated with the streptomycin-resistant strain, two groups of 40 guinea pigs each were inoculated intraperitoneally with 0.5 mg. of tubercle bacilli; one group received the control strain A27 and the other received the streptomycin-resistant strain. These groups were subdivided into 2 groups of 20 each, one of which served as control and the other was treated with streptomycin intramuscularly for a period of 12 weeks. Treatment was begun the day after infection. The drug was administered in 10 mg. per Kg. doses twice daily 5 days a week with a double dose on the fifth day. At 13 weeks the survivors were tuberculin tested with .01 mg. PPD intracutaneously. The experiment was terminated at 97 to

TABLE 2

Pathogenicity of streptomycin-resistant strains A27 in guinea pigs

EXPERIMENT NUMBER	TUBERCULOSIS STRAIN A27	NUMBER OF TRANSFERS IN STREPTOMYCIN	NUMBER OF GUINEA PIGS INOCULATED	DOSE INOCULATED INTRAPERITONEALLY	NUMBER OF ANIMALS DIED BEFORE TERMINATION OF EXPERIMENT	INTERVAL BETWEEN INOCULATION AND AUTOPSY	AVERAGE TUBERCULOSIS INDEX FOR THE GROUP
				mg.		*days*	
1	Streptomycin-resistant	Fifth transfer in 0.5 γ per cc.	17	1.0	1	42	10
	Control	—	17	1.0	1	42	11
2	Streptomycin-resistant	Seventh transfer in 0.5 γ per cc.	18	1.0	5	43	10
	Control	—	18	1.0	4	43	16
3	Streptomycin-resistant	Twelfth transfer, last 3 in 3.5 γ per cc.	19	0.25	0	42	8
	Control	—	18	0.25	1	42	10
4	Streptomycin-resistant	Sixteenth transfer, last 4 in 4.0 γ per cc.	20	0.5	12	97 to 107	9
	Control	—	20	0.5	16		12

106 days after inoculation, when the survivors were killed and the extent of tuberculous involvement noted and rated as previously described (10).

In figure 6 are shown the weight curves of the 4 groups of guinea pigs. As previously pointed out, the weight curve of the untreated animals which were inoculated with the resistant strain (curve R) was consistently higher than that of the untreated animals inoculated with the control strain (curve C). The weight curve of the group infected with the control strain and treated with streptomycin (curve CT) was the highest of the 4 groups. At 11 weeks the average weight of the animals of this group was 537 Gm. while that of the untreated was 363 Gm. Both groups infected with the resistant strain, whether treated or not treated with

streptomycin, had a somewhat higher weight curve than the untreated group infected with the control strain.

The slight drop in weight at 13 weeks was probably a result of the intracutaneous administration of .01 mg. of PPD. Of the 10 animals given PPD in the un-

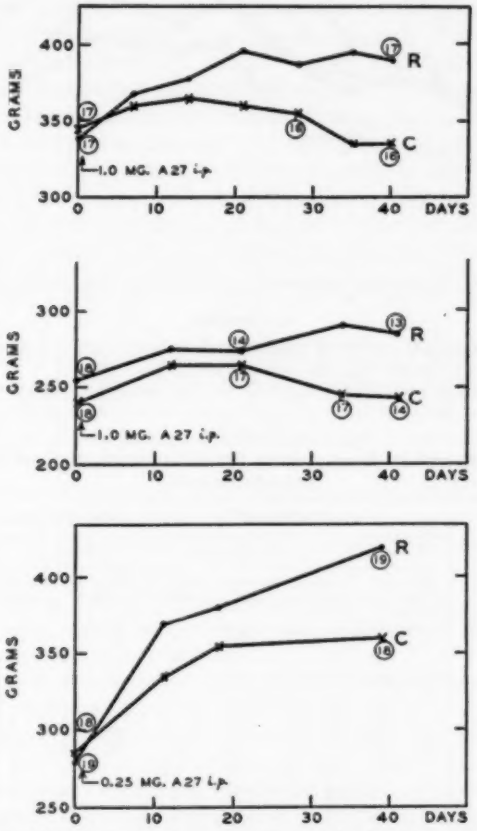


FIG. 5. Average weight curves of guinea pigs inoculated with the streptomycin-resistant strain (A27-R) and the control strain (A27-C):
Experiment 1. (Top) After 5 transfers in 0.5 γ per cc. streptomycin.
Experiment 2. (Center) After 7 transfers in 0.5 γ per cc. streptomycin.
Experiment 3. (Bottom) After 12 transfers in 0.5 γ to 3.5 γ per cc. streptomycin (Table 2).

treated group inoculated with the resistant strain, 7 were positive; while of the 10 of the treated group, 8 gave a pronounced positive reaction. In the untreated control group, 4 of the 5 survivors gave positive reactions while all of the 17 in the treated group, which had received the control A27 strain, gave positive PPD reactions.

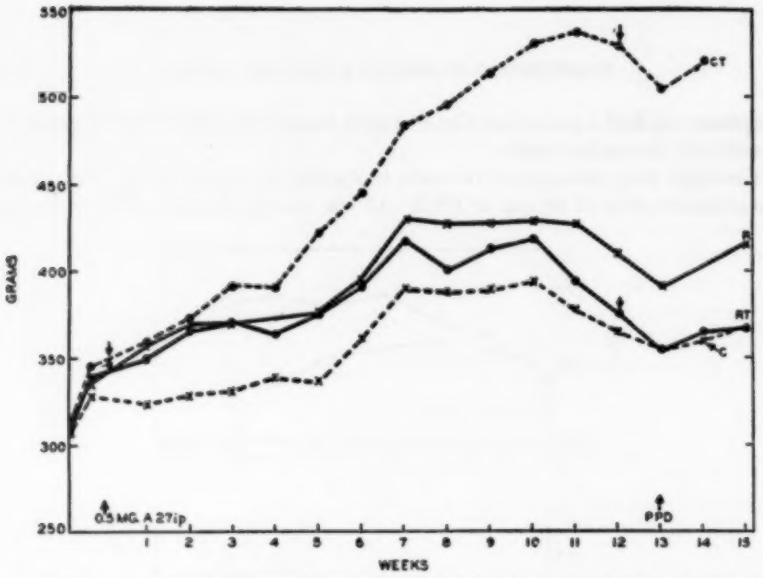


FIG. 6. Average weight curves of treated and untreated guinea pigs inoculated with streptomycin-resistant strain (A27-R) and control strain (A27-C).
Experiment 4. After 16 transfers in 0.5 to 4.0 γ per cc. streptomycin (Tables 2, 3).

TABLE 3

Virulence of streptomycin-resistant tubercle bacilli in guinea pigs and effect of treatment with streptomycin

GUINEA PIG NUMBER	CONTROL STRAIN A27				STREPTOMYCIN-RESISTANT STRAIN A27			
	Control		Treated		Control		Treated	
	Survived days	Tuberculosis	Survived days	Tuberculosis	Survived days	Tuberculosis	Survived days	Tuberculosis
1	47	10	98	1	K* 106	14	91	13
2	79	16	97	2	52	10	K 105	15
3	K 105	4	97	1	K 106	11	K 106	13
4	90	12	98	1	75	17	K 106	11
5	74	12	98	2	K 106	6	58	5
6	K 105	7	98	2	K 105	14	K 106	12
7	93	12	97	0‡	89	11	64	18
8	67	14	98	1	58	9	91	7
9	76	7	98	4	105	12	84	11
10	84	13	97	1	58	6	88	16
11	62	20	D† 33	3	K 106	5	61	6
12	81	12	D 89	4	90	14	95	17
13	74	11	98	2	89	15	36	10
14	K 105	5	D 12	0	K 106	4	K 106	8
15	75	11	97	3	K 106	4	K 106	19
16	K 105	15	98	1	84	9	90	10
17	74	18	98	5	K 105	3	K 106	11
18	37	15	98	4	105	6	92	12
19	83	19	97	3	83	9	K 105	16
20	77	11	98	2	58	5	89	14
Mortality per cent..	80		15		60		60	
Average tuberculosis index.....		12.2		2.2		9.2		12.2

* K—killed, † D—died, ‡ subculture of spleen negative after 35 days.

The survival time and the extent of tuberculous involvement for each animal of the 4 groups are given in table 3. At the termination of the experiment the mortality of the control group was 80 per cent while that of the group infected with the control strain and treated with streptomycin was 15 per cent. The mortality for both the streptomycin treated and untreated groups of guinea pigs which had been inoculated with the resistant strain was 60 per cent. The tuberculosis index of the guinea pigs inoculated with the A27 control strain was 12.2 while that of the control strain treated with streptomycin was 2.2. There was no difference in the tuberculosis index between the untreated group infected with the control strain and the treated group infected with the resistant strain. The untreated group infected with the resistant strain had a tuberculosis index of 9.2 while that of the treated group infected with the resistant strain was 12.2. The results of this experiment clearly indicate that treatment with streptomycin of tuberculous guinea pigs infected with the streptomycin-resistant strain of tubercle bacilli had no therapeutic effect on the course of the disease.

SUMMARY

1. The slow growing virulent human strain of tubercle bacilli (A27) has been made resistant to streptomycin by successive passages in Kirchner's medium with increasing concentrations of streptomycin. After 17 passages the degree of induced resistance was from an original sensitivity of 1.0 to 1.5 γ per cc. to a tolerance of 5.0 γ per cc.

2. The growth of the resistant strain in Kirchner's medium was slower when compared with the control strain. When the growth curves were subjected to statistical analysis it was found that the growth of the resistant strain was retarded on an average by 20 days.

3. The virulence of the resistant strain was indistinguishable from that of the control strain when tested by the short term technique of inoculation of the chorio-allantois of the chick embryo.

4. When tested in guinea pigs by intraperitoneal inoculation of 0.25 mg. to 1.0 mg. of tubercle bacilli and terminating the experiments 42 days after infection, the results of three tests showed a slightly reduced tuberculosis index and a higher average weight curve for the animals infected with the resistant strain. In a fourth experiment with 0.5 mg. of tubercle bacilli as the infecting dose with each strain, and the experiment terminated 14 to 15 weeks after infection, the mortality for the group infected with the resistant strain was 60 per cent as compared with 80 per cent for the control group and the tuberculosis index was 9.2 as compared with 12.2 for the controls.

5. Streptomycin therapy in doses which gave a high degree of protection in animals infected with the control strain was ineffective in infections with the resistant strain.

SUMARIO

Resistencia Adquirida del M. Tuberculosis a la Estreptomicina in vitro y Comportamiento del Mismo en las Infecciones Experimentales

1. La cepa humana de bacilos tuberculosos, virulenta y de desarrollo lento (A27) se convirtió en estreptomicinorresistente mediante pases sucesivos por el

medio de Kirchner con concentraciones crecientes de estreptomicina. Tras 17 pases, la intensidad de la resistencia inducida había pasado de la primitiva sensibilidad de 1.0 to 1.5 γ por cc. a una tolerancia de 5 γ por cc.

2. El crecimiento de la cepa resistente en el medio de Kirchner fue más lento que el de la cepa testigo. Al someter las curvas de crecimiento al análisis estadístico, observóse que el retardo del crecimiento de la cepa resistente promediaba veinte días.

3. La virulencia de la cepa resistente era indistinguible de la de la cepa testigo al comprobarla con la técnica breve de inoculación de la corioalantoides del embrión de pollo.

4. Al ensayar en los cobayos la inoculación intraperitoneal de 0.25 a 1.0 mg. de bacilos tuberculosos y terminar el experimento a los cuarenta y dos días de la infección, el resultado de tres ensayos reveló un índice tuberculoso algo menor y una curva ponderal media mayor para los animales infectados con la cepa resistente. En el cuarto experimento con 0.5 mg. de bacilos tuberculosos como dosis infectante con cada cepa, que terminó de catorce a quince semanas después de la infección, la mortalidad para el grupo infectado con la cepa resistente fué de 60 por ciento, comparado con 80 por ciento para el grupo testigo, siendo el índice tuberculoso de 9.2 y 12.2, respectivamente.

5. La estreptomicinoterapia, a dosis que suministraron una protección alta en los animales infectados con la cepa testigo, resultó ineficaz en las infecciones con la cepa resistente.

REFERENCES

(1) Youmans, G. P., Williston, E. H., Feldman, W. H., and Hinshaw, H. C.: Increase in resistance of tubercle bacilli to streptomycin: A preliminary report, Proc. Staff Meet., Mayo Clinic, 1946, *21*, 126.

(2) Williston, E. H., and Youmans, G. P.: Streptomycin resistant strains of tubercle bacilli, Am. Rev. Tubere., 1947, *55*, 536.

(3) Karlson, A. G., Feldman, W. H., and Hinshaw, H. C.: Persistence of resistance of tubercle bacilli to streptomycin during passage through guinea pigs, Proc. Soc. Exper. Biol. & Med., 1947, *65*, 6.

(4) Emmart, E. W.: The tuberculostatic action of streptothricin and streptomycin with special reference to the action of streptomycin on the chorio-allantoic membrane of the chick embryo, Pub. Health Rep., 1945, *60*, 1415.

(5) Emmart, E. W.: The tuberculostatic action of the sodium salts of certain synthetic alicyclic acids, Am. Rev. Tubere., 1946, *53*, 83.

(6) Bliss, C. I.: The dosage mortality curve, Ann. Applied Biol., 1935, *22*, 134.

(7) Irwin, J. O.: Statistical methods applied to biological assays, Supplement to J. Royal Statistical Soc., 1947, *4*.

(8) Deming, W. E.: Statistical Adjustment of Data, John Wiley and Sons, 1942, pp. 184, 197, Exp. 17.

(9) Emmart, E. W., and Smith, M. I.: The growth and effects of the tubercle bacillus on the chorio-allantoic membrane of the chick embryo: A method for studies in chemotherapy, Pub. Health Rep., 1941, *56*, 1277.

(10) Smith, M. I., Emmart, E. W., and Stohlman, E. F.: The action of some derivatives of 4-4'-diaminodiphenylsulfone in experimental tuberculosis, Am. Rev. Tubere., 1943, *48*, 32.

(11) Yegian, Diran, Budd, V., and Middlebrook, G.: Biologic changes in sulfonamide-*resistant mycobacterium ranae*, J. Bact., 1946, *51*, 479.

ROUTINE EXAMINATION OF SPUTUM FOR ACID-FAST BACILLI[1]

DAVID G. FREIMAN AND MAX PINNER[2]

(Received for publication May 11, 1948)

For the past several years the sputum and gastric contents of all patients admitted to this institution have been routinely examined by both microscopic and cultural methods. In an effort to determine the value of the methods used and the efficacy of the routine as a whole, an analysis has been made of the results obtained over a period of four years from January 1, 1940, when the routine was well established, to January 1, 1944. During this period, very few changes were made in the routine method, technical staff, or type of patient admitted. It has been possible, therefore, to make a fairly reliable evaluation of the relative performance of the various methods over a significant interval.

MATERIALS AND METHODS

Bacteriologic Procedure

The method of bacteriologic examination used routinely in this laboratory is as follows. Upon admission of the patient, three successive sputum specimens are sent to the laboratory as quickly as they can be collected in adequate amount, preferably within a period of ten days. These are examined for acid-fast bacilli by direct smear and, if negative, are concentrated. If all three specimens are negative for acid-fast bacilli, the third specimen is routinely cultured. The second or even the first specimen may occasionally be cultured, however, if the clinical condition of the patient warrants. Following the third admission specimen, the sputum is examined every one or two months, or more often if necessary. If the concentrate is persistently negative for tubercle bacilli, it is routinely cultured. If the sputum is repeatedly negative for tubercle bacilli, or if little or no sputum is available, the gastric contents are examined and all such specimens are cultured. Guinea pig inoculation is used only in special instances, usually in cases in which the roentgenographic appearance of the lesion seems to belie the persistently negative bacteriologic findings.

All smears for microscopic examination are stained individually by the Ziehl-Neelsen method and are examined intensively for a period of not less than five minutes. Concentrates are made by the addition of an equal volume of 3 per cent sodium hydroxide to the sputum or gastric specimen and incubating with frequent shaking for one to two hours. Cultures are made on a minimum of four tubes of the modification of Petragagni's medium described in Diagnostic Standards[3]. About 0.5 cc. of neutralized digest are inoculated into each tube by means of a capillary pipette and the tubes are incubated for a period of not less than two months before being discarded. All positive cultures are smeared for confirmation.

Composition of Material Subjected to Analysis

In the evaluation of the results of this analysis, several points must be borne in mind. The patients admitted to this institution are a rather highly selected

[1] From the Montefiore Hospital Country Sanatorium, Bedford Hills, New York.

[2] Doctor Pinner died on January 7, 1948.

[3] Diagnostic Standards and Classification of Tuberculosis, published by the National Tuberculosis Association.

group and include a large proportion of minimal and moderately advanced infections with a favorable prognosis. A significant number represent cases discovered on mass survey or on army induction examinations. Patients in the terminal stages of the disease are either not admitted or are quickly transferred. This practice is reflected in the very low number of sanatorium deaths, which average only one or two per year and often result from causes other than tuberculosis. It is not possible, therefore, to extend these results except in a very general way to institutions where all types of patients are freely admitted, or to the average office practice.

It must also be borne in mind that the results obtained in evaluating the various methods of examination represent not the actual efficiency of the method but rather its general contribution within the routine procedure described. Each patient received an unspecified number of sputum and gastric examinations conditioned by his physical status, therapeutic regimen, and length of stay, and no single specimen was subjected to all methods of examination. In actual practice, the selective methods routinely employed tend to weight the figures in favor of the microscopic methods. For example, sputum specimens found positive for acid-fast bacilli on direct smear tend to be repeatedly re-examined by this method and are rarely cultured, whereas specimens repeatedly negative are usually concentrated and cultured immediately without prior resort to smear. Furthermore, whereas all cultured sputum specimens are first concentrated and examined microscopically, only 31.9 per cent of concentrated sputum specimens are subsequently cultured. Since the uncultured specimens are those in which the presence of tubercle bacilli can most readily be expected on the basis of past performance and the clinical and roentgenographic appearance of the lesion, in other words, those in which a relatively high percentage of positive cultures is likely to be obtained, the microscopic method is thus favored. In the case of the gastric specimens a more reliable relationship obtains, since practically all specimens are subjected to both concentration and culture.

Results of the Analysis

The performance of the various methods of examination is shown in table 1. In spite of the fact that the microscopic methods are favored, the important contribution of the cultural method is evident. In the case of sputum specimens, culture is still nearly twice as effective in detecting tubercle bacilli as is microscopic examination of the concentrate; in the case of gastric specimens, culture is five times as effective. The significance of these figures is indicated by the relative constancy of these relationships for the four yearly intervals.

The value of the cultural method as an aid in bacteriologic examination for acid-fast bacilli is even more clearly shown in table 2. Of nearly 5,000 sputum and gastric specimens found to be negative for acid-fast bacilli by microscopic examination, an increment of 30 per cent was positive on culture. Furthermore, the remarkably small variation of this percentage when calculated for the various six month intervals is a significant commentary, both on the constancy of performance of technical personnel and culture medium and on the relatively

TABLE 1

Results of the various methods of examination for acid-fast bacilli

YEAR	SPUTUM										GASTRIC						
	Smear			Concentrate			Culture			Ratio: Per cent positive culture/Per cent positive concentrate	Concentrate			Culture			Ratio: Per cent positive culture/Per cent positive concentrate
	Total	Positive		Total	Positive		Total	Positive			Total	Positive		Total	Positive		
		Number	Per cent		Number	Per cent		Number	Per cent			Number	Per cent		Number	Per cent	
1940	606	367	60.5	1,893	326	17.2	520	208	40.0	2.3	586	26	4.4	586	160	27.3	6.2
1941	567	350	61.7	1,793	420	23.4	502	180	35.9	1.6	737*	43	5.8	735	221	30.1	5.2
1942	616	340	55.2	1,654	376	22.7	592	236	39.8	1.8	1,095*	85	7.8	1,079	268	24.8	3.2
1943	548	227	41.4	1,473	297	20.1	623	213	34.2	1.7	920*	38	4.1	912	215	23.6	5.8
Total.....	2,337	1,284	55.1	6,813	1,419	20.8	2,237	837	37.4	1.8	3,338	192	5.8	3,312	864	26.2	5.0

* A few gastric specimens strongly positive on concentrate were not cultured.

constant character of the patient group during the period of study. The slight decline in percentage over the four year period is probably a result of the gradual increase in the number of accidentally discovered cases, a trend also noticeable in table 1.

A more reliable index of the relative efficacy of the different methods of detecting tubercle bacilli can be obtained by determining which method was responsible for the first detection of acid-fast organisms in each patient admitted. The processes of selection inherent in the routine method employed again introduce a significant error since the first two specimens examined are usually sputum and are rarely cultured. A positive culture, therefore, frequently implies failure of at least three microscopic examinations.

TABLE 2

Efficiency of culture method: Results of culturing sputum and gastric specimens which were negative on concentrate

YEAR		TOTAL SPECIMENS CULTURED	POSITIVE ON CULTURE	
			Number	Per cent
1940	January to June	438	158	36.1
	July to December	554	165	29.8
1941	January to June	553	167	30.2
	July to December	541	171	30.8
1942	January to June	769	221	28.8
	July to December	704	191	27.1
1943	January to June	732	212	28.0
	July to December	673	184	27.4
Total		4,964	1,469	29.6

The results of this analysis are shown in table 3, which again reveals the importance of the cultural method. Of 611 patients with pulmonary tuberculosis admitted to this institution during a four year period, and whose sputum or gastric content was found to be positive for acid-fast bacilli at some time during the sanatorium stay, 65.8 per cent were first found to be positive by a microscopic method, while 34.2 per cent were first found by culture. This represents an increment by culture of about 50 per cent, since in virtually all these cases the microscopic methods had failed to detect the organism. Examination by cultural methods is thus responsible for the first positive finding in one-third of the cases found positive by all methods employed. Concentration methods account for an additional third, and direct smear the remainder. In this connection it

should be noted that examination of sputum by direct smear in this institution reveals acid-fast bacilli in only one-third of the patients with pulmonary tuberculosis in spite of the fact that great precautions are taken to choose a satisfactory sputum particle for smear.

The results presented in table 3 also serve to emphasize the importance of the examination of the gastric contents in the routine search for tubercle bacilli. It may be seen that 22.9 per cent, or almost one out of every four cases, is found to be positive for the first time by this method. Gastric examinations are frequently performed after repeated examination of the sputum, often with culture as well as concentration, has been unsuccessful, or when little or no sputum is available. Thus a large proportion of minimal and treated cases is included. By far the largest number of cases first found to be positive for acid-fast bacilli by examination of the gastric contents is discovered by culture and the overall increment by microscopic examination of the concentrate (5.2

TABLE 3

Analysis of type of examination which resulted in first detection of acid-fast bacilli

YEAR OF ADMISSION TO SANATORIUM	NUMBER OF PATIENTS FOUND TO BE POSITIVE BY SOME METHOD DURING SANATORIUM STAY	SPUTUM						GASTRIC			
		Smear		Concentrate		Culture		Concentrate		Culture	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
1940	160	57	35.7	45	28.1	29	18.1	8	5.0	21	13.1
1941	158	53	33.6	40	25.3	32	20.2	5	3.2	28	17.7
1942	158	49	31.0	48	30.4	17	10.8	13	8.2	31	19.6
1943	135	49	36.3	29	21.5	23	17.0	6	4.5	28	20.7
Total.....	611	208	34.0	162	26.6	101	16.5	32	5.2	108	17.7
				471	77.1					140	22.9

per cent) is so small as to be hardly worth the time spent in studying the slides. It has become a routine practice, therefore, to examine microscopically only those gastric specimens which can reasonably be expected to be positive on clinical and roentgenographic grounds, or on the basis of previous bacteriologic examinations.

All methods combined account for positive findings in only 86.2 per cent of the patients studied; in the remaining 13.8 per cent all specimens were negative on repeated examination (table 4). Unfortunately, guinea pig inoculations were not done on most of these cases but, wherever done, were also negative. This group of 98 cases is analyzed in table 5. About one-third (35 per cent) are patients in whom effective collapse therapy had been instituted prior to admission and whose sputum was presumably positive for tubercle bacilli before such therapy was begun. An additional third consists of patients with inactive disease, as evidenced by serial roentgenograms (28 per cent), and a few cases of pleural effusion without demonstrable parenchymal infiltration (5 per cent). A

few patients whose lesions were definitely proved to be nontuberculous and several patients with sarcoidosis of the lungs have not been included. Only a relatively small proportion of these negative cases (32 per cent), therefore, consists of patients with active disease, and of these the majority are of minimal extent and/or show very slight roentgenographic evidence of instability. If the other

TABLE 4

Incidence of cases persistently negative during sanatorium stay

YEAR	ADMISSIONS*	CASES FOUND POSITIVE BY SOME METHOD DURING SANATORIUM STAY		CASES PERSISTENTLY NEGATIVE DURING SANATORIUM STAY	
		Number	Per cent	Number	Per cent
1940	179	160	89.4	19	10.6
1941	179	158	88.3	21	11.7
1942	189	158	83.6	31	16.4
1943	162	135	83.3	27	16.7
Total.....	709	611	86.2	98	13.8

* Patients proved to be nontuberculous or those in whom the bacteriologic workup could not be considered adequate are excluded.

TABLE 5

Analysis of cases bacteriologically negative throughout sanatorium stay

YEAR OF ADMISSION TO SANATORIUM	CASES PERSISTENTLY NEGATIVE DURING SANATORIUM STAY*	ACTIVE DISEASE: LESIONS ROENTGENOLOGICALLY UNSTABLE DURING SANATORIUM STAY					EFFECTIVE COLLAPSE THERAPY INSTITUTED PRIOR TO ADMISSION				INACTIVE DISEASE: LESIONS ROENTGENOLOGICALLY STABLE DURING SANATORIUM STAY		PLEURAL EFFUSION WITHOUT DEMONSTRABLE PARENCHYMAL INFILTRATION	
		Total		Extent			Total		Type					
		Number	Per cent	Min.	Mod. Adv.	Far Adv.	Number	Per cent	Pneumothorax	Thoracoplasty	Number	Per cent	Number	Per cent
1940	19	4	21	4	0	0	11	58	10	1	4	21	0	—
1941	21	6	29	4	0	2	8	38	6	2	4	19	3	14
1942	31	8	26	6	2	0	8	26	6	2	14	45	1	3
1943	27	13	48	5	7	1	7	26	5	2	6	22	1	4
Total...	98	31	32	19	9	3	34	35	27	7	28	28	5	5

* Cases proved to be nontuberculous are excluded.

groups are eliminated, these 31 patients represent only 4.8 per cent of the 642 patients with active pulmonary tuberculosis admitted to the sanatorium during the four year period. Acid-fast bacilli are thus demonstrable by microscopic or cultural methods in 95 per cent of patients in whom such a finding can reasonably be expected.

As it is reasonable to suppose that a certain proportion of the remaining 5 per

cent would have been found positive if a larger number of specimens had been examined, it is important from a practical standpoint to determine what procedures constitute a satisfactory bacteriologic investigation. The 611 positive cases were therefore analyzed to determine how many specimens had to be examined before a positive specimen was found by any method, and the results are tabulated in table 6. It will be noted that one-half (51.5 per cent) of the cases were found to be positive for acid-fast bacilli on the first examination. This usually included direct smear and concentrate, as well as a small proportion of gastric examinations (including culture) in those patients who either did not bring up sputum or had not yet learned how to do so. The high percentage of positive findings in the third specimen was largely a result of the fact that this specimen was cultured routinely if the preceding specimens were negative. Of particular significance is the fact that 82.5 per cent of the cases were found to be positive after three examinations (including at least one culture if the microscopic methods yielded negative results). Each subsequent examination added a very small increment. In all these respects the figures show a remarkable degree of constancy for the various yearly periods.

COMMENT

The importance of the culture method in examinations for acid-fast bacilli is clearly indicated by all the forms of analysis followed. In this institution the contribution of the use of culture was of a high order as it provided an increment of 30 per cent of positive findings in cases found negative for acid-fast bacilli by microscopic methods. Moreover, the use of cultures was the means of detecting the first acid-fast bacillus in one-third of the cases found positive by all methods combined. These particular values, however, are significant only within the routine framework employed and may not be extended to other situations without consideration of several variable factors.

The character of the patient group: The role played by culture becomes increasingly important as the number of bacilli in the specimen diminishes toward and beyond the limit of sensitivity of the microscopic method used. The present series includes a considerable number of patients with early and minimal lesions, as well as a significant number convalescing from surgical procedures such as thoracoplasty. Of this group only 66 per cent were first detected by the microscopic methods. In contrast to this, in a series of 507 patients analyzed by Pinner and Werner (1), over 60 per cent were classified as far advanced, and over 90 per cent were first found positive for acid-fast bacilli by the use of microscopic methods. Similarly, in 82 per cent of a series of 1,657 adult patients recently reported by Sanford (2), the first detection of acid-fast bacilli was made by microscopic methods. The death rate during the sanatorium treatment of this group was 34 per cent. Culture plays its greatest role in the identification of the relatively rare organisms in the pulmonary secretions of early, healing, and surgically treated cases, and therefore becomes progressively more important as the number of such cases increases in any patient group.

The quality of the microscopic methods: The performance of the culture methods

serves in some measure as a criterion of the quality of the smear and concentrate as well as of the efficiency of the culture. Since an increment by culture of 30 per cent (table 2) represents positive specimens which were not detected by the microscopic methods, it is reasonable to assume that an increase in the quality and efficiency of these methods should reduce the percentage. The various refinements aimed at accomplishing this purpose are directed either toward examination of a larger amount of the sample, or toward improvement of concentration and staining techniques. In institutional work, at least, all such refinements must be evaluated by weighing the additional time expended in the examination against the increase in positive findings that can be expected.

One of the methods of increasing the amount of the sample examined is the preparation and examination of more than one smear. Such a study was made in this laboratory using a series of 508 sputum concentrates. Duplicate smears were made and each was intensively examined for five minutes, thus doubling the usual examining time. The results, as presented in table 6, show an increment of 12 per cent in the percentage of specimens positive for acid-fast bacilli. Pottenger (3) has made an analysis of results obtained by examining sputum smears for increasing periods of time. Of sputum specimens microscopically positive after a fifteen minute search, 67 per cent were positive after five minutes and 90 per cent after ten minutes. This represents an increment of about 25 per cent for the second five minutes, or about twice that found in the duplicate smear series. It should be noted, however, that the criteria of positivity are not entirely comparable as culture is used in one series and microscopy in the other. In any event, the results of culture indicate that at least 45 per cent of the sputum concentrates examined are actually positive for tubercle bacilli, representing an increment about four to seven times as great as either method alone (table 6). Furthermore, in institutional practice at least, increasing the number of specimens examined tends to be more rewarding than increasing the time expended on each individual sample regardless of whether one or multiple smears are used.

Improvements in concentration technique have been limited by the necessity for retaining viable tubercle bacilli for subsequent culture. For this reason, most of the suggested improvements employing various hydrocarbon detergents cannot be readily utilized. Of the numerous modifications of staining technique that have been suggested, most of them offer little or no real advantage over the Ziehl-Neelsen method. A promising exception, however, appears to be the fluorescence method. In our own hands (4) little advantage was observed with the possible exception of the very thin gastric concentrate smears, but this appears to have resulted in large measure from an inefficient light source (5). More recent reports have been more enthusiastic (6).

The efficiency of the culture media: The number of suggested improvements in culture media are too numerous to detail here. Of the several tried in this laboratory, none so far suggested appears to offer much advantage over the modified Petragagni used. In spite of the disadvantages of malachite green as an inhibiting dye (7) it has, in our hands, offered the most satisfactory balance be-

TABLE 6

Analysis of number of specimens examined before first detection of acid-fast bacilli (all methods combined)

YEAR OF ADMISSION TO SANATORIUM	NUMBER OF PATIENTS FOUND TO BE POSITIVE AT SOME TIME DURING SANATORIUM STAY	FIRST ACID-FAST BACILLUS FOUND ON SPECIMEN NUMBER																	
		1		2		3		4		5		6		7		8		9 or more	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
1940	160	82	51.2	24	15.0	24	15.0	7	4.4	6	3.8	5	3.1	0	—	5	3.1	7	4.4
1941	158	84	53.1	15	9.5	32	20.2	8	5.1	6	3.8	5	3.2	5	3.2	0	—	3	1.9
1942	158	82	51.9	28	17.7	25	15.8	6	3.8	9	5.7	3	1.9	3	1.9	0	—	2	1.3
1943	135	67	49.6	20	14.8	21	15.5	10	7.4	5	3.7	4	3.0	2	1.5	2	1.5	4	3.0
Total...	611	315	51.5	87	14.2	102	16.7	31	5.1	26	4.3	17	2.8	10	1.6	7	1.2	16	2.6
		504	82.5																

tween growth of tubercle bacilli and the inhibition of contaminating organisms. Some index of the efficiency of the medium is provided by culture of specimens known to be positive for acid-fast bacilli on microscopic examination. Of 261 such specimens 221, or 84.7 per cent, showed growth. On the other hand, of all cultures planted, 5.1 per cent were discarded because of gross contamination.

There is obviously considerable room for improvement in the culture medium: in increasing the number of positive specimens; in reducing the number of contaminations; and in reducing the time required to produce visible growth. The synthetic medium containing water soluble esters of long chain fatty acids described by Dubos and others (8, 9, 10) appears to be a hopeful step in this direc-

TABLE 7

Results obtained by doubling the examination time compared with those to be expected by culture

	TOTAL SPECIMENS EXAMINED	POSITIVE AFTER FIVE MINUTES		POSITIVE AFTER TEN MINUTES		INCREMENT BY DOUBLING TIME OF EXAMINATION	POSITIVE ON CULTURE		INCREMENT BY CULTURE OVER 5 MINUTE EXAMINATION
		Number	Per cent	Number	Per cent		Number	Per cent	
						per cent			*per cent*
Sputum concentrates examined by duplicate smear method	508	126*	25	141†	28	+12	239‡	47	+88
Sputum concentrates total for four year period	6,813	1,419	21	1,890§	28	+25	3,015‡	44	+110

* Figure represents number of positive findings on first smear examined.

† Figure represents number of positive findings on at least one of the two smears.

‡ Calculated, representing number of specimens positive after five minutes plus 30 per cent of the remaining negative specimens; the latter represents the average expected increment by culture as shown in table 2.

§ Calculated, representing the approximate number of positive findings to be expected after ten minutes based on the figures of Pottenger (3).

tion. In its present form, however, it is not readily adaptable for routine use, especially in view of the frequency of contamination.

Time and frequency of use of culture methods: It is obvious that earlier and more frequent use of culture in any routine procedure tends to increase the number of positive findings and to reduce the average number of specimens required to demonstrate acid-fast bacilli. Thus, earlier use of culture would cause a "shift to the left" in table 5; its elimination would tend to produce a "shift to the right;" the examination of more specimens would be necessary before the same results are obtained No generalization as to the optimal time and frequency of use of culture can be made but, in view of the fact that such a large proportion of the first two specimens examined is found to be positive microscopically, earlier routine use of culture, in institutional work at least, is wasteful of time and culture media far out of proportion to any advantage gained. Modifications of the

routine can be readily made, however, if the laboratory is kept informed of existing problems, and close cooperation between laboratory and clinic may save a great deal of time and effort on both sides.

SUMMARY

An analysis of the results of routine examination for acid-fast bacilli of sputum and gastric contents over a four year period clearly demonstrates the important contribution of the cultural method within the routine framework employed. Culture is at least two to five times as effective as the microscopic methods in spite of selective weighting of the figures. In addition, it provides an increment of 30 per cent of positive findings in specimens found negative by the microscopic methods used and is a means of first detecting acid-fast bacilli in one-third of cases found positive by all methods combined. The importance of culture of gastric contents is emphasized, this method accounting for the first positive finding in 23 per cent of cases. All of these figures show remarkable constancy for each of the yearly periods. Of those cases found positive at some time during their hospital stay, 82 per cent were revealed by the first three examinations. Of all 709 patients studied, 14 per cent remained negative on repeated examination. Two-thirds of these patients, however, had either been previously treated or were roentgenologically inactive. If these cases are eliminated, acid-fast bacilli were demonstrated by microscopic and/or cultural methods in 95 per cent of patients in whom such a finding could be reasonably expected.

SUMARIO

Examen Sistemático del Esputo en Busca de Bacilos Acidorresistentes

Un análisis de los resultados obtenidos con el examen sistemático del esputo y el contenido gástrico en busca de bacilos acidorresistentes durante un cuatrienio demuestra claramente el importante aporte del método cultural en relación con el sistema empleado. El cultivo resulta a lo menos dos a cinco veces más eficaz que las técnicas microscópicas a pesar de la valuación selectiva de las cifras. Además, suministra un incremento de 30 por ciento de hallazgos positivos en los ejemplares que resultan negativos con las técnicas microscópicas usadas y ofrece un medio de distinguir los primeros bacilos ácidorresistentes en la tercera parte de los casos que resultan positivos con todas las técnicas combinadas. Recálcase la importancia que revisten los cultivos del contenido gástrico, correspondiendo a esta técnica el primer hallazgo positivo en 23 por ciento de los casos. Todas estas cifras muestran notable constancia para cada uno de los períodos anuales. De los casos que resultaron positivos en alguna época durante su hospitalización, 82 por ciento fueron descubiertos durante los primeros tres exámenes. De los 709 enfermos estudiados, 14 por ciento permanecieron negativos a través de exámenes repetidos; pero dos terceras partes de éstos o bien habían sido tratados antes o eran radiográficamente negativos. Eliminando estos casos, se encontraron bacilos ácidorresistentes con las técnicas microscópicas y/o culturales en 95 por ciento de los enfermos en los que cabía razonablemente esperar dicho resultado.

Acknowledgment

The authors wish to thank Dr. Gabriele Bondi for her aid in analyzing the negative cases.

REFERENCES

(1) Pinner, M., and Werner, W. I.: The significance of positive and negative sputum findings in pulmonary tuberculosis, Am. Rev. Tuberc., 1928, *18*, 490.

(2) Sanford, L. L.: Cultures of pulmonary secretions: Their accuracy in the evaluation of activity in pulmonary tuberculosis, Am. Rev. Tuberc., 1946, *54*, 67.

(3) Pottenger, J. E.: The importance of the time of search in examining stained preparations for rare tubercle bacilli, J. Lab. & Clin. Med., 1931, *16*, 985.

(4) Freiman, D. G., and Mokotoff, G. F.: Demonstration of tubercle bacilli by fluorescence microscopy: A comparative evaluation of the fluorescence and Ziehl-Neelsen methods in the routine examination of smears for acid-fast bacilli, Am. Rev. Tuberc., 1943, *48*, 435.

(5) Graham, C. F.: Fluorescence microscopy of Mycobacterium tuberculosis: A study of basic factors, Am. Rev. Tuberc., 1943, *48*, 421.

(6) Levinson, L., and White, R. L.: The detection of tubercle bacilli by fluorescence microscopy, New England J. Med., 1947, *237*, 186.

(7) Corper, H. J., and Cohn, M. L.: Contrast coloring of media by dyes in growing tubercle bacilli: Effect of dyes on growth of tubercle bacilli, Am. J. Clin. Path., 1946, *16*, 621.

(8) Dubos, R. J.: Rapid and submerged growth of Mycobacteria in liquid media, Proc. Soc. Exper. Biol. & Med., 1945, *58*, 360.

(9) Dubos, R. J., Davis, B. D., Middlebrook, G., and Pierce, Cynthia: The effect of water soluble lipids on the growth and biological properties of tubercle bacilli, Am. Rev. Tuberc. 1946, *54*, 204.

(10) Foley, G. E.: Submerged growth of tubercle bacilli from pathologic material in Dubos' medium, Proc. Soc. Exper. Biol. & Med., 1946, *62*, 298.

A STUDY OF THE BACTERIOSTATIC AND BACTERICIDAL ACTIVITY OF PROMIN, DIASONE, SULFATHIAZOLE, SULFAGUANIDINE AND STREPTOMYCIN ON THE H-37V. STRAIN OF M. TUBERCULOSIS[1]

K. T. SASANO

(Received for publication January 14, 1948)

INTRODUCTION

The trials that have been conducted in the chemotherapeutic treatment of tuberculosis in the past few years have revealed that only partial success is at hand. It is evident that it is not enough to obtain clinical cure through the use of antibiotic or other therapeutic drug even if the clinical improvement is dramatic. Because of the nature of the pathology of tuberculosis, it seems that any chemotherapeutic agent to be most effective in tuberculosis must have bacteriostatic as well as bactericidal properties. The exact action of the substances which have been used therapeutically in recent years is not clearly known and there has been insufficient work done on these substances in the laboratory, especially in relation to their bactericidal properties on *M. tuberculosis*. The present report concerns an investigation of the bacteriostatic and bactericidal properties of promin, diasone, sulfathiazole, sulfaguanidine, and streptomycin. The strain of *M. tuberculosis* of human origin H-37v. was the one chosen for the study.

In these studies the bacteriostatic effect was determined by the growth of the bacilli in a liquid culture medium to which various amounts of the drug to be tested were added to a series of culture tubes. The culture media used consisted of a modified Proskauer and Beck medium and the Dubos Tween-albumin medium. The growth of the bacilli was determined after twenty days of incubation at 37.5°C.

RESULTS

The bacteriostatic effect of the drugs tested is shown in table 1. It will be noted that streptomycin was the most, and sulfaquanidine the least, effective bacteriostatic agent of the drugs tested. In another experiment not shown in the table it was found that the addition of penicillin to the streptomycin dilutions did not materially enhance the tuberculostatic effect of streptomycin.

From these experiments it would seem that streptomycin and sulfathiazole gave the greatest promise for a possible favorable chemotherapeutic effect.

The bactericidal effect of the various compounds was determined by exposing a suspension of tubercle bacilli which contained one mg. (wet weight) of organisms per cc. of normal saline solution to different concentrations of the substance to be tested.

At one, twenty-four and forty-eight hour intervals after the bacilli were brought in contact with the drug, one cc. of the bacillary suspension was removed and washed in 20

[1] From the laboratory of J. C. R. S. Sanatorium, Spivak, Colorado.

volumes of normal saline solution by centrifugation for twenty minutes at 3,000 r.p.m. Cultures were made from the centrifuged sediment which was resuspended in the original volume. Guinea pigs were inoculated with 0.5 cc. of the washed bacillary suspension. Cultures and guinea pig inoculations were made from a control bacillary suspension which contained the same amount of tubercle bacilli but no drug. The cultures on egg-yolk-potato medium were examined at three weeks and the guinea pigs were killed on the day when the control animals died.

The results of these experiments are presented in table 2. As may be seen in the table, it was found that promin was distinctly bactericidal in solution of 100

TABLE 1

Bacteriostatic effect of various drugs on growth of tubercle bacilli in modified Proskauer and Beck broth (20 days incubation)

CONCENTRATION OF DRUGS	STREPTOMYCIN	SULFATHIAZOLE	PROMIN	DIASONE	SULFAGUANIDINE
mg. per 100 cc.					
100	—	xxxx	xxxx	xxxx	xxxx
50	—	xxxx	xxxx	xxxx	xxx
40	—	xxxx	xxxx	xxx	xxx
30	—	xxxx	xx>	xxx	xx
20	—	xxxx	xxx	xx	x
15	—	xxxx	xx	x	0
10	—	xxxx	x	0	0
5	—	xxxx	0	0	0
2.5	—	xxx	0	0	0
1	xxxx	x	0	0	0
0.1	xxxx	—	—	—	—
0.05	xxxx	—	—	—	—
0.02	xx	—	—	—	—
0.01	x	—	—	—	—
0.005	0	—	—	—	—
Control	0	0	0	0	0

Explanation of symbols (bacteriostatic effect):
xxxx = Complete bacteriostasis; no growth on culture medium.
xxx = Trace of growth.
xx = Poor growth.
x = Good growth.
0 = Heavy growth.
— = No test.

mg. per 100 cc. and diasone in 333 mg. per 100 cc., whereas the other compounds revealed no bactericidal effect. Streptomycin on the other hand reduced the number of colonies as determined by culture but did not kill all the organisms. In another experiment not shown in the table the addition of 100,000 units of penicillin per 100 cc. of the bacillary suspension did not alter the bactericidal effect of streptomycin.

The relative bacteriostatic and bactericidal properties as determined by *in vitro* studies and by animal inoculations on a streptomycin-sensitive strain of tubercle bacillus are presented in table 3.

TABLE 2

Bactericidal effect of various drugs on tubercle bacilli (cultures and animal inoculations were done with washed bacillary suspensions)

CONCENTRATION	DRUGS	CULTURE AFTER HOURS OF CONTACT WITH DRUGS			GUINEA PIGS INOCULATED WITH
		1 hr.	24 hrs.	48 hrs.	0.5 cc. of 48 hr. sample
mg. per 100 cc.					
1,000	Streptomycin	—	xx	xx	x
500	Promin	0	xxxx	xxxx	xxxx
	Diasone	0	xxxx	xxxx	xxxx
400	Streptomycin	—	xx	xxx	x
333	Promin	0	xxxx	xxxx	xxxx
	Diasone	0	xxxx	xxxx	xxxx
250	Promin	0	xxxx	xxxx	xxxx
	Diasone	0	xx	xxx	xx
200	Streptomycin	—	xx	xx	x
100	Promin	0	xxx	xxxx	xxxx
	Diasone	0	x	xx	xx
	Sulfathiazole	0	0	0	0
	Sulfaguanidine	0	0	0	0
	Streptomycin	—	x	xx	x
Bacillary control suspension		0	0	0	0

Explanation of symbols (bactericidal effect):

xxxx = No growth on egg yolk medium: no tuberculous lesion in guinea pigs.

xxx = One or two colonies on culture: only local or lymph node lesion in guinea pigs.

xx = Few colonies on culture: moderate number of tuberculous lesions in spleen and liver.

x = Good growth on culture: generalized tuberculosis but not so extensive as in controls.

0 = Heavy growth on culture: massive generalized tuberculosis in guinea pigs.

— = No test.

TABLE 3

Comparative bacteriostatic and bactericidal efficiencies of various drugs on M. Tuberculosis

DRUGS	BACTERIOSTATIC CONCENTRATION IN MG. PER 100 CC.		BACTERICIDAL CONCENTRATION IN MG. PER 100 CC.
	Minimal inhibition	Complete inhibition	48 hours exposure to drugs
Promin	10	40	100
Diasone	15	50	333
Sulfathiazole	1	5	Absent
Sulfaguanidine	20	100	Absent
Streptomycin	0.01	0.05	?

(?)—Some bacilli were killed but some were viable after exposure to all concentrations; 10:1,000 mg. per 100 cc.

DISCUSSION

It is well known that the therapeutic efficacy of many drugs does not correspond necessarily with their bacteriostatic or bactericidal activity as found in *in vitro* studies. This is true in some of the sulfonamide compounds studied. For example, sulfathiazole manifested bacetriostatic activity *in vitro* which was eight times that of promin. Nevertheless, sulfathiazole was not bactericidal for *M. tuberculosis* when tested in concentrated solution for forty-eight hours and had no therapeutic effect on experimental tuberculosis in guinea pigs when 100 mg. were given daily. Diasone was found to possess less bacteriostatic and bactericidal potency than promin *in vitro*, but was almost as effective as promin in the treatment of tuberculous guinea pigs. According to Ballon and Gueron (1), the growth of *M. tuberculosis* was inhibited in a concentration of 100 mg. per 100 cc. of promin and in 10 mg. per 100 cc. of sulfathiazole. In the writer's laboratory the growth of *M. tuberculosis* H-37v. was completely inhibited in synthetic broth containing either 40 mg. per 100 cc. of promin or 5 mg. per 100 cc. of sulfathiazole. This difference may have resulted from the method of sterilization or from differences in the culture media employed. A few colonies of *M. tuberculosis* have been observed in a modified Herrold medium containing 50 mg. per 100 cc. of promin. The streptomycin exhibited the highest degree of bacteriostasis for it completely inhibited the growth of *M. tuberculosis* in a concentration of 0.05 mg. per 100 cc. Streptomycin appears to have little bactericidal effect, however, for it did not kill all the bacilli when tested in a concentration of 1,000 mg. per 100 cc. for forty-eight hours. The present observations agree with Middlebrook and Yegian (2) and Wolinsky and Steenken (3) relative to the bacteriostatic effect of streptomycin. The combination of penicillin and streptomycin in the proportion of one unit to one microgram, respectively, did not appear to have any influence on the effect of streptomycin. Since streptomycin is bacteriostatic in high dilution and is not bactericidal even in high concentration, it would seem reasonable to expect that a smaller dosage of the antibiotic could be used without sacrificing the therapeutic effect provided the tubercle bacilli remain sensitive. Fisher (4) found that cultures from one-half of patients treated with streptomycin showed various degrees of resistance to the antibiotic after 84 to 126 days of therapy. Cultures obtained from the other patients remained sensitive to streptomycin concentrations of one γ per cc. or less. In the J. C. R. S. Sanatorium streptomycin-resistant strains of tubercle bacilli have been cultured from over 70 per cent of the treated cases.

The problems that have arisen as the result of the therapeutic application of various chemotherapeutic agents in human tuberculosis suggest that the bactericidal, as well as the toxic and bacteriostatic properties of any new compounds, be determined by *in vitro* and *in vivo* laboratory studies prior to application to man. It may well be that a bacteriostatic agent without bactericidal properties may influence the appearance of resistant strains of organisms during a course of treatment.

SUMMARY

From laboratory studies with a streptomycin-sensitive strain of *M. tuberculosis* H-37v. it has been found that: (*1*) promin and diasone possess both bacterio-

static and bactericidal properties, with promin the more active of the two; (*2*) sulfathiazole possesses bacteriostatic activity to a high degree without appreciable bactericidal effect; (*3*) sulfaguanidine has much less bacteriostatic effect than sulfathiazole and is not bactericidal; (*4*) streptomycin exhibits the greatest bacteriostatic effect but has only slight bactericidal activity; (*5*) the bacteriostatic effect of streptomycin is not altered by the presence of penicillin.

SUMARIO

Estudio, con la Cepa H-37v del Bacilo Tuberculoso, de las Propiedades Bacteriostáticas y Bactericidas de la Promina, la Diasona, el Sulfatiazol, la Sulfaguanidina y la Estreptomicina

Por los estudios de laboratorio ejecutados con una cepa estreptomicinosensible del bacilo tuberculoso H-37v, se ha descubierto que: (1) la promina y la diasona poseen propiedades tanto bacteriostáticas como bactericidas, siendo la promina la más activa de las dos; (2) el sulfatiazol muestra alta actividad bacteriostática sin apreciable efecto bactericida; (3) la sulfaguanidina ejerce mucho menor efecto bacteriostático que el sulfatiazol y no es bactericida; (4) la estreptomicina manifiesta el mayor efecto bacteriostático pero apenas posee actividad bactericida, y (5) el efecto bacteriostático de la estreptomicina no es alterado por la presencia de penicilina.

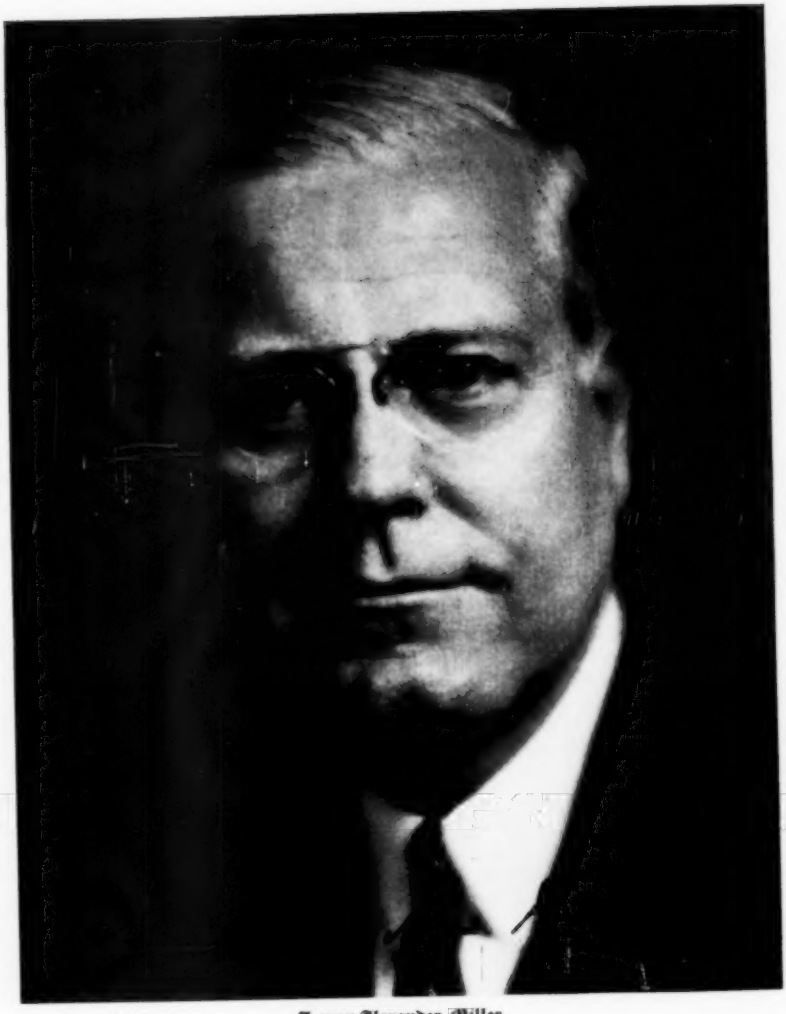
REFERENCES

(1) Ballon, H. C., and Gueron, A.: The effect of sulfathiazole and allied compounds on tubercle bacilli in vitro, Am. Rev. Tuberc., 1942, *45*, 212.

(2) Middlebrook, G., and Yegian, D.: Certain effect of streptomycin on *mycobacterium in vitro*, Am. Rev. Tuberc., 1946, *56*, 553.

(3) Wolinsky, E., and Steenken, W., Jr.: Effect of streptomycin on the tubercle bacillus: The use of Dubos and other media in test for streptomycin sensitivity, Am. Rev. Tuberc., 1947, *55*, 281.

(4) Fisher, M. W.: Streptomycin resistant tubercle bacilli, Am. Rev. Tuberc., 1948, *57*, 53.



James Alexander Miller
1874–1948

James Alexander Miller
1874-1948

Dr. James Alexander Miller, a former president of the National Tuberculosis Association and the New York Tuberculosis and Health Association, died on the 29th of July, 1948, at the age of seventy-four.

After completing his preliminary education in Princeton University and receiving his medical degree from the College of Physicians and Surgeons of Columbia University in 1899, he entered upon the practice of medicine in New York City, continuing until the time of his death. Starting in 1901, he also practiced for eight summers at Paul Smith's in the Adirondacks, and it was here that he cultivated a long lasting friendship with Dr. Edward Livingston Trudeau and acquired the inspiration to concentrate much of his later professional activity in the field of tuberculosis. During the subsequent years he extended his interest into other sides of medicine and public health, but always with particular focus on the unique problems of this specific disease. His growing experience and the time tested attributes of his personality, intellect, and character qualified him as an able leader and marked him for succession to many posts of responsibility and importance. His merits included a love of the truth, high ideals, devotion to study, patience, perseverence, self-discipline, generous consideration of his associates, and easy adaptability.

Doctor Miller's interest and accomplishments in the campaign against tuberculosis were not defined by any narrow limits, since he was a man of versatility and naturally endowed with broad vision. Early in his career he became interested in the Tuberculosis Service, now the Chest Service, of Bellevue Hospital, which at the time hardly deserved the name of a clinical service. Through the ensuing years he succeeded in creating a competent organization which raised the standard of medical work to its present level. Recognizing the numerous backgrounds of tuberculosis, he interested philanthropic laymen in the social problem, leading to the organization of an Auxiliary which raised funds from private sources and looked after many of the needs of the impoverished patients. He became Professor of Clinical Medicine in the College of Physicians and Surgeons of Columbia University and developed a course of instruction for medical students and for the house staff of the hospital which continues to claim the interest of many young physicians at the start of their careers. In the 1920's Doctor Miller obtained funds to initiate research as a feature of the Service; this too has grown and continues to the present date. This connection with Bellevue brought him into contact with many governmental officials who respected his judgment and advice, and it was largely through his personal efforts and influence that the present building housing the Bellevue Chest Service was planned and constructed.

The public health aspects of tuberculosis also commanded his interest and energy. Aside from his work in the national and local tuberculosis associations, he was an officer or adviser in many organizations, including the Community

Service Society, the Milbank Memorial Fund, the Commonwealth Fund, and the New York Academy of Medicine where, for many years, he was chairman of the Committee on Public Health, now Public Health Relations. His counsel was often followed because of his unusual ability to visualize problems in concrete terms, to plan the logical and practical approach, and to set in motion the machinery for solution. In action he never spared himself and it was largely for this reason that he was so successful in obtaining the cooperation of others. In his large private practice it was his humanity which impressed so many patients.

The wide recognition of Doctor Miller's abilities and accomplishments was indicated in part by his election to the presidency of such medical organizations as the American College of Physicians, the American Climatological and Clinical Association, the Practitioner's Society, the New York Medical and Surgical Society, and the New York Academy of Medicine. From 1927 to 1945 he also served as President of the Board of Trustees of the Trudeau Sanatorium. He continued as Visiting Physician in Charge of the Tuberculosis Service of Bellevue Hospital until 1938, following which he was Honorary Consultant. He was honored with Doctor of Science degrees by Columbia (1930) and Princeton (1936) and with a Doctor of Public Health degree by New York University (1937). In recognition of his work with the Red Cross and the Rockefeller Commission for the control of tuberculosis in France during World War I, he was made a Chevalier of the Legion of Honor. The National Tuberculosis Association awarded him the Trudeau Medal in 1944 and the New York Academy of Medicine, its Academy Medal in 1947. On this occasion, marking its Centennial Celebration, the honor was bestowed upon him as "the Academy's most distinguished and beloved Fellow and one of the greatest benefactors of mankind."

J. Burns Amberson

THE

AMERICAN REVIEW OF TUBERCULOSIS ABSTRACTS

VOLUME 59 APRIL, 1949 ABST. No. 4

Bronchial Stenosis after Pneumothorax.—The authors report 3 cases of pulmonary tuberculosis in which stenosis of the left main bronchus developed about two and a half years after the beginning of pneumothorax treatment. These constrictions within the bronchus were smooth, no ulceration being present. Biopsies showed they were composed of fibrous tissue without characteristic microscopic signs of tuberculosis. Clinically the patients had at first improved under pneumothorax treatment but eventually had suffered from recurrent febrile attacks following retention of sputum and dilatation of the bronchus below the constriction. In one case multiple areas of softening developed throughout the collapsed lung. In another a subapical cavity increased in size. In the third the constriction was not followed by changes in the collapsed lung. It was possible in each case to locate the constriction by tomography and confirm its presence by bronchoscopy. The use of lipiodol was considered inadvisable. The stenoses were believed to be due to a sclerosing type of bronchial tuberculosis aided by mechanical factors. One of these may have been the narrowing of the lumen of the left bronchus by congestion of its network of arterial, venous and lymphatic vessels following pneumothorax. Such congestion could result ultimately in fibrosis. This form of stenosis can be considered a clinical entity which can be diagnosed by the presence of a smooth constriction of the left main bronchial stem, developing a year or two after the induction of pneumothorax and followed by febrile episodes with alternate retention and outpouring of sputum. Tomography is very useful in detecting and locating the constriction. As regards treatment, discontinuance of the pneumothorax is not always indicated since a lung blocked by stenosis will not reexpand and lack of air in the pneumothorax area may cause serious symptoms. Thoracoplasty is usually contraindicated. Dilatation of the stenosis may succeed but the only logical treatment is complete removal of the involved lung.—*Réflexions sur les sténoses des bronches souches sous pneumothorax artificiel, G. Giraud, A. Balmes, L. Nichet & J. Mirouze, Le Poumon, September-October, 1947, 3: 329.*—(*A. T. Laird*)

Bronchial Foreign Bodies.—In dealing with translucent foreign bodies diagnostic roentgenograms should be overpenetrated and the correct position determined by fluoroscopic examination. Both inspiratory and expiratory films should be made to show mediastinal shift. Proper roentgenograms may show a distinct interruption of the air column of the involved bronchus.—*Some observations on the roentgen diagnosis of nonopaque foreign bodies aspirated into the bronchi, S. Welin, Acta radiol., 1948, 29: 529.*—(*J. E. Farber*)

Metallic Breath Sounds.—Metallic breath sounds are usually heard over a pneumothorax space when inspiratory and expiratory pressures are both positive. Occasionally they can also be heard over a pneumothorax with negative pressures. A certain tension of the pneumothorax wall is necessary for the production of these metallic breath sounds and this tension can be due either to a positive pressure pneumothorax or to changes in the

structure of the chest wall itself due to inflammation, fibrosis, or shrinkage. Metallic sounds over a closed pneumothorax are faint and loud metallic sounds suggest the presence of a valvular pneumothorax or of a very large cavity in the lung. Metallic sounds may develop rapidly over a negative pressure pneumothorax when a dry pleurisy causes increased tension of the pneumothorax wall.—*Metallie über dem Pneumothoraxraum-kein obligates Ueberdrucksymptom, K. Bauer, Wien, klin. Wchnschr., December 12, 1947, 59: 811.*—(*G. C. Leiner*)

Accidental Extrapleural Pneumothorax.—Accidental extrapleural pneumothorax may occur during the course of therapeutic intrapleural pneumothorax as described in 1933 by Corbetta. Oeschsli in 1938 reported 15 instances among 400 pneumothorax cases. The author describes 7 cases seen within two years. The roentgenologic appearance is striking with gas in the extrapleural space. This gas is very slowly absorbed and may cause pain in the hypochondrium with a tight feeling in the chest. The extrapleural cavities may be confused with adhesions so that the pneumothorax is sometimes stopped. Manometric readings may be confusing and in themselves lead to abandoment of the pneumothorax. Finally, the extrapleural pocket may enhance the formation of adhesions by approximating the pleural surfaces.—*Accidental extrapleural pneumothorax, O. Olsson, Acta radiol., 1948, 28: 117.*—(*J. E. Farber*)

Level of Diaphragm in Emphysema.—The level of the diaphragm is of clinical importance in the diagnosis of emphysema. Radiological aids for this diagnosis are (*1*) position of the ribs, (*2*) translucency of the lung fields, and (*3*) the level of the diaphragm. None of these has been determined accurately and quantitatively. The level of the diaphragm was measured in this work. The distance between a line joining the iliac crest and the level of the highest point of the diaphragmatic dome on each side on full inspiration and full expiration was measured, as suggested by Warner and Doidge. This can be determined more accurately by screening at 3 ft. distance than by films, since under the fluoroscope one can check that the subject has actually reached the limit of inspiration or expiration. The quotients:

$$\frac{\text{distance from lowest diaphragmatic level to iliac crest}}{\text{distance from tuber ischii to acromion process}}$$

and

$$\frac{\text{distance from highest diaphragmatic level to iliac crest}}{\text{distance from tuber ischii to acromion process}}$$

were established. Altogether 58 subjects were examined, including 8 who were normal, 5 obese normal, 7 with heart disease who had recently suffered from congestive failure but were not in failure at the time, and 38 with clinical emphysema. These results show what was to be expected, namely, that the level of the diaphragm in pronounced emphysema is always lower than normal. In slight emphysema a tendency toward a low diaphragm is unmistakable although the level may be within normal limits. The only exceptions are the obese emphysematous patients, in whom the diaphragm would have to overcome the higher resistance of the more voluminous abdomen. It is possible that this resistance prevents the lowering of the diaphragm. Any tendency to increase the volume of the lung would, in these cases, result in the lifting of the ribs only. This is known to happen in many cases of emphysema apart from the diaphragmatic changes. The following mean values were secured using the aforementioned quotients (first figure is "low level" of diaphragm, second figure is "high level"): normals, 0.35, 0.47; slight emphysema, 0.31, 0.44; moderate and severe emphysema, 0.27, 0.34; obese emphysema, 0.37, 0.47; obese normals, 0.36, 0.48; cardiac patients recovered from congestive failure, 0.36, 0.44.—*Radiological determination of the level of the diaphragm in emphysema, M. Grossmann & H. Herzheimer, Brit. J. Radiol., September, 1948, 21: 446.*—(*B. Hyde*)

Loeffler's Syndrome with Cavity Formation.—A 39 year old woman developed several pulmonary infiltrations within a few weeks, with fever and a blood eosinophilia of 30 per cent. The infiltrations resorbed promptly but one of them showed cavity formation in the centre. This cavity healed within three weeks.—*Ueber ein zerfallendes, eosinophiles, flüchtiges Lungeninfiltrat, A. Ott, Wien. klin. Wchnschr., May 21, 1948, 60: 320.*—(*G. C. Leiner*)

Pleural Effusion.—The object of this study was to estimate the significance of early pleural effusion in pulmonary embolism. In a series of 46 cases, 21 were found to have a pleural effusion. In more than one-half of these cases the pleural effusion was more evident roentgenographically than the parenchymal density. However, the presence of the effusion was of little help in diagnosing pulmonary embolism. In a series of 38 cases of pneumonia and bronchopneumonia only a few developed pleural effusion and the parenchymal density was the principal radiographic finding. Pleural effusions are sometimes seen in acute, non-specific respiratory tract infections without parenchymal lesions or with only small lung densities.—*Early pleural effusion in pulmonary embolism and pneumonia or bronchopneumonia, G. Moberg, Acta radiol., 1948, 29: 7.*—(*J. E. Farber*)

Spastic Bronchitis.—Bronchitis spastica without real asthmatic attacks has been seen more frequently recently than in previous years, possibly as a consequence of the war. The dyspnea which is found in spastic bronchitis can always be relieved by epinephrine. Bronchospasm is always seen in patients with emphysema and is frequent in cardiac patients.—*Ueber Bronchospasmus, W. Gröger, Wien. klin. Wchnschr., January 23, 1948, 60: 49.*—(*G. C. Leiner*)

Mediastinal Hernia.—Mediastinal hernias may be caused by pressure-difference in the two hemithoraces at the 2 weak places described by Nitsch. Hernias caused by pulsion (pneumothorax) are demonstrated by fluoroscopy. Hernias caused by traction are best shown by tomography. The diagnostic signs seen in the tomograms are the upper borderline of the hernia and the visualization of the blood vessels crossing from the healthy hemithorax into the pouch of the hernia. (Author's Summary)—*The roentgen examination of the mediastinal lung hernia with reference to tomography, H. Salinger, Acta radiol., 1948, 29: 130.*—(*J. E. Farber*)

Thoracic Aortography.—The author reports a new technique for visualization of the thoracic aorta. A catheter is introduced into the radial artery and guided under radioscopic control via the subclavian artery into the ascendent aorta. After the rapid injection of 30 cc. of diodrast, exposures of the aorta are made. (Author's Summary)—*Thoracal aortography by catheterization from the radial artery, S. Radner, Acta radiol., 1948, 29: 178.*—(*J. E. Farber*)

Thoracic Aortography.—A method for aortography suggested by Radner and somewhat modified has been used in 3 cases. The authors consider that the method should be preferred to angiocardiography at contrast roentgen examinations of the aorta and its intra-thoracic branches. (Authors' Summary)—*Thoracic aortography, B. Brodén, H. E. Hanson & J. Karnell, Acta radiol., 1948, 29: 181.*—(*J. E. Farber*)

Lung Changes in Dermatitis.—In 2 patients with an acute generalized dermatitis roentgenograms of the lung showed an increased reticular pattern. As the skin lesion cleared, the abnormal lung changes disappeared. The author suspects hypersensitivity as the cause of both lesions with changes probably in the interstitial tissue.—*On lung changes in acute general dermatitis, N. Frostberg, Acta radiol., 1948, 29: 493.*—(*J. E. Farber*)

Mediastinal Abscess.—Roentgenologic diagnosis of a mediastinal abscess can some-

times be aided by mediastinal puncture and injection of a contrast medium. The risk of such a puncture is reduced by using a very fine needle and the procedure is much easier if performed under fluoroscopy. In the case reported, the patient was given a little barium to swallow to simplify the localization. The needle was inserted into the right supraclavicular fossa in a downwards and medial direction reaching the abscess wall. The cavity was aspirated and penicillin was injected with subsequent improvement.—*Mediastinal abscess diagnosed by roentgen, S. R. Kjellberg, Acta radiol., 1948, 30: 294.—(J. E. Farber)*

Tracheal Diverticula.—Multiple tracheal diverticula may be demonstrated by bronchography, as occurred in one patient being studied for bronchiectasis. Besides the diverticula, cylindric and saccular bronchiectasis was found in both lower lobes.—*A case of multiple tracheal diverticula, K. Nielsen, Acta radiol., 1948, 30: 331.—(J. E. Farber)*

BCG and Tuberculosis Diagnosis.—Following a BCG vaccination the tuberculin test gives a positive reaction and, therefore, cannot be used further as an aid to differential diagnosis. A Russian method of avoiding this difficulty was tested. In a group of 180 vaccinated children the Mantoux reaction was positive in 86 per cent; the Pirquet test, if performed simultaneously, was positive in 52.8 per cent. In another group of 180 vaccinated children the Pirquet test was done first and the Mantoux test was done two weeks later. There was a positive Mantoux reaction in 86 per cent of the children but a positive Pirquet reaction in only 5.8 per cent. None of the 360 children had pathological changes in the sedimentation test or the roentgenologic examination. It is believed that the Mantoux and Pirquet tests, if done at a certain interval, will be of use in the differential diagnosis. A positive Pirquet reaction after BCG vaccination does not mean the presence of a reinfection nor is reinfection excluded by a negative Mantoux and a negative Pirquet reaction.—*Experimentelle Beiträge zu den in Zusammenhang mit der B. C. G.—Impfung auftauchenden differentialdiagnostischen Problemen, St. Zimányi, Wien. klin. Wchnschr., August 13, 1948, 60: 515.—(G. C. Leiner)*

Auto-urine Injections for Tuberculosis.—Eight patients with pulmonary tuberculosis received intramuscular injections of their own fresh urine. The treatment was started with 0.5 cc., the injections being given every two or three days. The dose was increased every time by 0.5 cc. to a final volume of 5 cc. No improvement of the pulmonary condition of the patients was seen.—*Ergebnisse der Eigenharnbehandlung nach Plesch bei der Lungentuberkulose, Beyrer, Wien. klin. Wchnschr., July 2, 1948, 60: 418.—(G. C. Leiner)*

Ultrasonic Waves and Tubercle Bacilli.—Human tubercle bacilli can be damaged by exposure to ultrasonic waves so that they stop multiplying. These tubercle bacilli no longer cause tuberculosis in the guinea pig but, if injected, they produce some immunity to subsequent infection with virulent tubercle bacilli. It can be expected that tubercle bacilli which have been exposed to ultrasonic waves will be used for prophylactic vaccination of human beings. *Ueber die Wirkung von Ultraschall auf Tuberkulosekeime von Typus humanus and die Möglichkeit einer Schutzimpfung gegen die Tuberkulose, F. Kress, Wien. klin. Wchnschr., September 17, 1948, 60: 597.—(G. C. Leiner)*

Pulmonary Edema.—The roentgenologic picture of pulmonary edema, acute and chronic, has only recently been described. The heart is enlarged and the lung fields contain symmetrical, confluent, perihilar ill-defined patches of increased density. The central distribution of the opacities leaving the apices, peripheral borders, and bases relatively clear is characteristic. The bronchi stand out clearly whereas the vascular markings are not seen. The differential diagnosis includes

bronchopneumonia or multiple hemorrhagic infiltration of pleura and lungs. In the three cases of verified pulmonary edema presented the roentgenographic changes appeared earlier than the clinical signs.—*On the roentgenologic picture of pulmonary edema, S. Rennoes, Acta radiol., 1948, 30: 169.*—(*J. E. Farber*)

Tuberculoproteins. I.—Different preparations of purified protein derivative and Old Tuberculin are known to differ in their skin activity. Since this property is associated only with protein, a partial explanation of this phenomenon is that the protein content of certain lots of Old Tuberculin may be significantly different but this can hardly account for the variation found in preparations of PPD. The presence of more than one protein or the degradation of the protein during preparation might account for the observed facts. Concentrated, unheated culture filtrates of two strains of human tubercle bacilli, a virulent and a slightly virulent one, were fractionated with ammonium sulphate to give 14 fractions from each strain. Chemical determinations and sedimentation velocity measurements were made on those fractions for which significant results could be obtained. The evidence showed that two distinct proteins are present in addition to a polysaccharide and nucleic acid. The physical measurements did not demonstrate the presence of any other proteins. One of the proteins was isolated in pure form and found to have a molecular weight of 44,000 ± 5,000, as determined by the partial specific volume, sedimentation velocity, and diffusion rate. This protein is believed to be the one previously isolated by Seibert et al., who assigned it a molecular weight of 32,000. The other protein was not freed from polysaccharide so that its molecular weight could not be determined although it is believed to have a sedimentation constant of about 2 S. Sedimentation and diffusion constants were obtained for the polysaccharide which appears to be a homogeneous molecular species with a molecular weight of about 20,000.—*The proteins in unheated culture filtrates of human tubercle bacilli I. Fractionation and determination of physical-chemical properties, E. B. Bevilacqua & J. R. McCarter, J. Exper. Med., March, 1948, 87: 229.*—(*J. S. Woolley*)

Tuberculous Droplet Infection in Rabbits.—Experiments have been reported in which rabbits were made to inhale virulent bovine tubercle bacilli, air-borne as separated cells in fine drop nuclei under standardized conditions. The tubercles which developed in their lungs were known to be induced by organisms derived from single cells, all of which were implanted upon alveolar tissue within relatively brief intervals. For the first five or six weeks the rate of tubercle development was found to be uniform and not appreciably affected by the number of the lesions, their position in the lungs, or by the varying susceptibility of the rabbits. This initial, homogeneous phase contrasted sharply with the later stages of the infection which were strikingly heterogeneous especially when rabbits of various strains were used. These features of air-borne tuberculosis are now reported in more detail. The Ravenal strain of *Mycobacterium tuberculosis bovis* was prepared in an aqueous suspension which was whirled into a fine mist in a special aerosol flask. Only the smallest droplets evaporated rapidly enough to produce the droplet nuclei of which the final aerosol consisted. Occasional droplet nuclei contained tubercle bacilli. The concentration of the organisms in the aerosol and the length of exposure determined the intensity of infection. Fifty-six albino rabbits were exposed in these experiments and killed at various intervals. During the first week after infection the bacilli were always found in isolated alveolar macrophages. Between the sixth and ninth days the number of infected macrophages rapidly increased and at twelve days there were collections of parasitized macrophages occupying one or two alveoli. At this time leucocytes and small monocytes had begun to accumulate about the developing tubercles. A brief, early nonspecific response to uninfected droplet nuclei also was seen. By the

end of the third week typical tubercles became visible. During the fifth week differences in the rate of progress of the tubercles from animal to animal were detectable although all lesions were remarkably uniform in size in the lungs of any one animal. By the end of the sixth week of infection individual differences in the rate of progress of the initial tubercles and of the infection as a whole became evident. No evidence of delayed tubercle formation was encountered. This heterogeneity of the pattern of the disease seemed to be related to the intensity of the inflammation which in turn seemed to correspond to the differences in the growth rate of the bacilli after the fourth week, these differences being estimated by the number of organisms found in the lesions. It appears that heterogeneity of progressive tuberculosis may be related to differences in the capacity of animals to change the composition of their tissue or blood in such a way that growth of the bacilli may be more or less inhibited. It is concluded that rabbits do not differ in their lack of resistance to initial growth of bovine tubercle bacilli. However, the later, heterogeneous pattern of response suggests that these animals vary widely in their capacity to acquire resistance.—*Tuberculosis of rabbits induced by droplet nuclei infection: I. Initial response to infection, H. L. Ratcliffe & W. F. Wells, J. Exper. Med., June, 1948, 87: 575.*—(*J. S. Woolley*)

Apical Lung Cancer.—Experience with 92 cases of the Pancoast-Tobias tumor is presented. Early diagnosis can be made by roentgenograms and the first sign to be observed is a horn-like or semilunar density covering the pleural dome. Tomography helps to separate atelectasis from the tumor shadow. Several tumors varying both histologically and in location were found to produce the syndrome.—*Sur la pathologie et le diagnostic radiologique du cancer apical pulmonaire et des tumeurs malignes péri-apicales, R. Mathey-Cornat & de Fleurian, Acta radiol., 1948, 29: 19.*—(*J. E. Farber*)

Traumatic Paralysis of Diaphragm.—A 51 year old man fell from a ladder, fractured and dislocated his right clavicle, and fractured the upper seven ribs on the right. The right diaphragm was paralyzed, apparently by compression of the phrenic nerve between the fractured clavicle and the lateral processes of the cervical spine.—*Traumatische Zwerchfellähmung mit Totalverrenkung des Schlüsselbeins, R. Hagen, Wien. klin. Wchnschr., September 24, 1948, 60: 624.*—(*G. C. Leiner*)

Hering-Breuer Reflex.—The minimal pressure required for a reflex respiratory response during pentothal inhibition may be used to measure the rate of recovery of excitability. Reflex respiratory responses to light pressure on the chest wall in the deeply anesthetized dog are abolished by bilateral cervical vagotomy. Unilateral cervical vagotomy or unilateral pneumonectomy enhances the reflex response of the deeply anesthetized dog when lying on the unoperated side; when the operated side is down the reflex response is depressed. The dog with a unilateral vagotomy or unilateral pneumonectomy has a faster respiratory rate when lying on the unoperated side than when on the operated side. This difference in rate with change in position persists indefinitely in the unanesthetized animal. (Authors' summary).—*Role of the Hering-Breuer reflex under deep pentothal anesthesia, J. C. Scott, E. A. Reed, D. Saris & H. P. Redondo Ramirez, Am. J. Physiol., September, 1948, 154: 428.*—(*G. C. Leiner*)

Rib Anomaly.—The chest roentgenogram of a 25 year old female showed an unusual distal bifurcation of the fourth rib on the right. This bifurcation caused a constriction of the right hemithorax, a pleural adhesion, and the development of a septum running through the middle and lower lobes of the lung. This is the so-called "bifid rib of Luschka".—*Ueber eine ungewöhnliche rippenanomalie, zugleich ein Beitrag zur distalen Rippengabelung, P. Lutz, Wien. Klin. Wchnschr., December 7, 1947, 59: 846.*—(*G. C. Leiner*)

Tracheal Foreign Body.—The author describes a previously unreported sign of foreign

bodies in the trachea in children. There is inspiratory collapse below and simultaneous widening of the air passages above the foreign body. These changes disappear on expiration.—*A previously unreported sign of foreign bodies in the trachea, S. R. Kjellberg, Acta radiol., 1948, 30: 300.*—(*J. E. Farber*)

Thymic Shadow on Chest Roentgenogram.—This report is based on 498 consecutive roentgenograms of children first taken when they were six months old and subsequently at six month intervals. A peculiar sail-like, triangular projection was noted extending out from the mediastinum in 44 children (8.8 per cent). In 12 patients the shadow was so large that it obscured almost the whole of the upper lung field. There were 12 children with large shadows, 12 with medium sized shadows, and 20 with small shadows. In 31 cases the shadow was on the right side, in 12 cases it was on the left, and in one there was a small bilateral projection of which the right was the larger. In some cases the mediastinum was displaced towards the side of the shadow. Lateral roentgenograms were not of value but oblique films indicated that the shadow was anterior. In many cases, the lower edge of the shadow on the right side coincided with the plane of the horizontal fissure. Eleven of these children were followed for two years or more, during which time the shadows gradually diminished in size and in some cases disappeared. Almost all these children were in good health when roentgenographed, only 4 being ill. None had any symptom or sign of pulmonary or cardiac disease and postmortem correlation indicated that this shadow was actually thymus gland. In an infant, who died following a splenectomy for an obscure form of hemolytic anemia, the chest roentgenogram taken at autopsy revealed the previously described shadow on the right side. After the sternum had been removed but before the organs were disturbed, surgical clips were placed on the poles of the thymic lobes and a repeat chest roentgenogram was taken. It was found that the clips corresponded exactly with the outlines of the shadow seen on the first roentgenogram. In stillborn children, the lateral limits of the thymus extend farther out than when the lungs are inflated. It was suggested that, as the lungs expand, the thymus is stripped from the chest wall and compressed into the mediastinum, but that sometimes one or both lower poles are adherent so that it remains anchored in the position occupied in the fetus. This theory was supported by the observation that in some cases with a unilateral shadow the mediastinum shifted toward that side. As the child grows older, the thymus is gradually stripped back until the shadow ultimately disappears. The finding of this sail-like, triangular projection from the mediastinum (thymus) does not have any pathologic significance.—*A sail-like triangular projection from the mediastinum: A radiographic appearance of the thymus gland, F. H. Kemp, H. N. C. Morley & E. Enrys-Roberts, Brit. J. Radiol., December, 1948, 21: 618.*—(*L. Hyde*)

Roentgenograms in Sarcoid.—The roentgenological aspect of sarcoidosis is discussed. Lung films may show enlargement of mediastinal nodes, miliary infiltration, areas of fibrosis, and confluent areas of patchy infiltration. Bilateral mediastinal lymphadenopathy is common but unilateral involvement occurs. Similarly, unilateral parenchymal lesions occur but also less often than bilatetal lesions. Pulmonary manifestations are rarely uniform; no single type of infiltration can be described as an early or late phase of the disease. Reisner, however, believes that the disseminated nodular forms are early lesions. Skeletal changes occur in 20 per cent of the patients. The bone lesions resemble small cysts and are found in the phalanges of the fingers and toes.—*Roentgenological aspect of sarcoidosis, A. J. Ackerman, Am. J. Roentgenol., March, 1948, 59: 318.*—(*J. E. Farber*)

Retrocardiac Bronchiectasis.—Bronchiectasis involves the lower lobe of the left lung more frequently and more extensively than any other pulmonary area. The occurrence of unilateral bonchiectasis is nearly five times as

frequent in the left as in the right lung. Isolated bronchiectasis of the retrocardiac area of the left lower lobe is frequent. On routine chest roentgenograms it may escape recognition entirely and can be diagnosed only by bronchographic examination.—*Retrocardiac bronchiectasis, R. G. Bloch, L. F. Sandock & E. B. Mitchell, Am. J. Roentgenol., August, 1948, 60: 219.*—(*J. E. Farber*)

Streptomycin in Tuberculosis Surgery.—Prior to the use of streptomycin, pulmonary resections for tuberculosis carried a mortality rate of 25 per cent and an incidence of empyema of 12 per cent and of bronchopleural fistula of 8 per cent. In most places, the incidence of spread following thoracoplasty was 3 to 6 per cent. In 28 cooperating hospitals every other tuberculous patient undergoing thoracoplasty and every patient with tuberculosis undergoing resection received streptomycin. The daily dosage at first was 2.0 Gm.; later this was reduced to 1.0 Gm. The drug was given for one week before and two weeks after each operation. In a total of 1,347 thoracoplasty stages, streptomycin was given for 699 operations on 258 patients. Postoperative spreads or reactivations appeared in 14 (2 per cent) and wound infections in 8 patients. In the control group, there was spread in 5.6 per cent and a correspondingly larger number of wound infections. The reduction was felt to be significant, but other factors such as improved technique, anesthesia, et cetera, may have contributed. Therefore, the routine administration of streptomycin in thoracoplasty is not recommended; it should be given in selected cases. A total of 129 resections were done, including 77 lobectomies and 52 pneumonectomies. There was a mortality rate of 4.5 per cent and an incidence of empyema of 4 per cent. These results are so striking when compared to the previous figures as to indicate that the use of streptomycin in pulmonary resection is mandatory.—*Streptomycin in surgery of pulmonary tuberculosis, J. D. Murphy, Surg., Gynec. & Obst., November, 1948, 87: 546.*—(*A. G. Cohen*)

Streptomycin for Tuberculosis.—Streptomycin was used in the treatment of 225 tuberculous patients after August, 1947, at the Hôpital Laennec in Paris. The average daily dose was 1.5 Gm. Skin eruptions appeared in 22 per cent, nausea and vomiting in 34 per cent, and vestibular disturbances in 39 per cent. There was one case of deafness which appeared after three months of treatment. Renal function could not be adequately studied. Therapeutic results were as follows: Of 78 patients with meningitis, 31 were living after six to eight months. Among 54 cases of miliary tuberculosis with or without meningitis, there were 36 surviving patients, mostly those without meningitis. Roentgenograms showed complete clearing. Of 64 patients with pulmonary tuberculosis (nonmiliary), 70 per cent had regression of clinical symptoms (fever, weight loss, cough, expectoration). Regressive roentgenographic changes were most marked in the diffuse nodular infiltrations of recent date; old lesions showed no response. Pneumonic consolidations sometimes gave a good initial response but the effect was usually incomplete. Cavitary lesions were seldom influenced but good results were obtained by combining streptomycin with collapse therapy. Thirty-three cases of laryngeal and pharyngeal tuberculosis showed favorable results with healing of the tuberculous lesions in 12 cases and great improvement in 11 cases. Dysphagia disappeared with great rapidity; the effect on dysphonia was slower and less constant. Streptomycin broke up the parallelism usually existing between the laryngeal and pulmonary lesions. Regression of laryngeal pathology was observed with no change in the pulmonary status. In endobronchial tuberculosis, streptomycin had a favorable influence on edematous, granulomatous and freshly ulcerative forms.—*Rapport sur le traitement de la tuberculose par la streptomycine, E. Bernard & A. Lotte, with the collaboration of B. Kreis, J. S. Bourdin & G. Arnaud, Rev. de la tuberc., 1948, 12: 165.*—(*V. Leites*)

You get the

Contrast you want...

in your hospital with this

RADIOGRAPHIC RULE of THREE...

CONTRAST is under complete control . . . when you use x-ray products made by Kodak to work together. This combination—Kodak film, intensifying screens, and processing chemicals—in competent hands assures entirely predictable radiographic quality . . . a factor so essential in radiologic diagnosis.

1 *Use* **KODAK FILM— BLUE BRAND**

2 *Expose with* **KODAK SCREENS—** three types

3 *Process with* **KODAK CHEMICALS—** liquid or powder

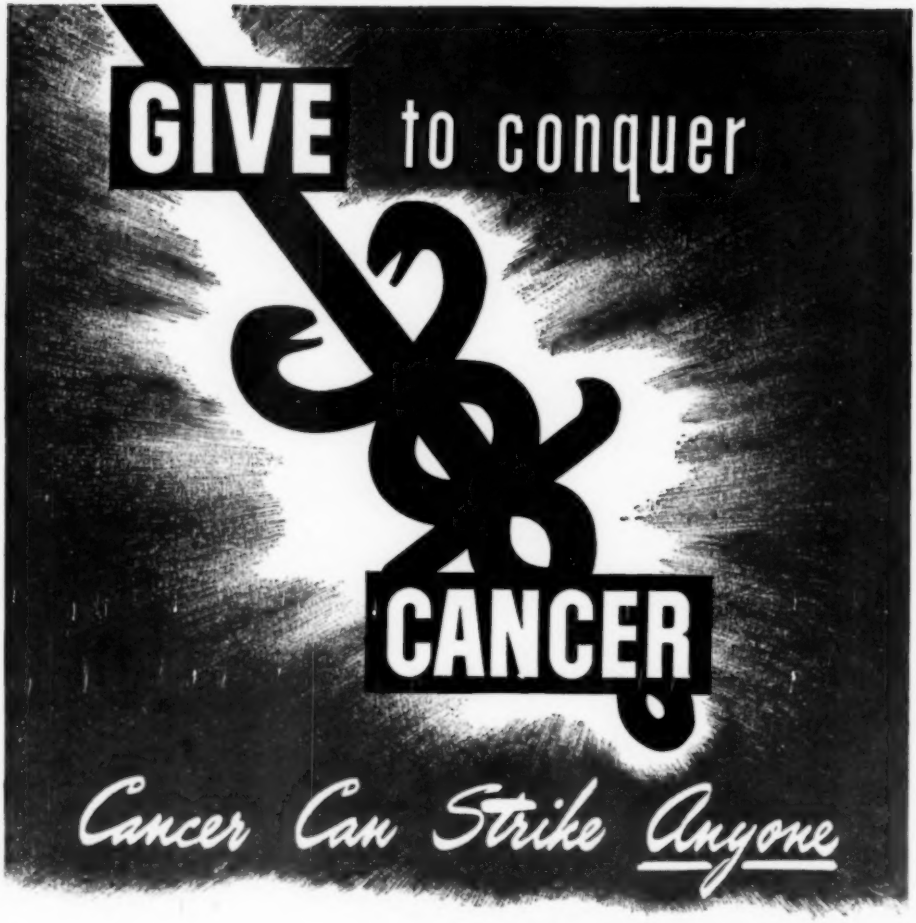
KODAK PRODUCTS FOR RADIOGRAPHY

Blue Brand and No-Screen Medical X-ray Films . . . Photoflure Films for photoradiography . . . Periapical and Occlusal Dental X-ray Films . . . Fine-Grain, High-Definition, and Ultra-Speed X-ray Intensifying Screens . . . Exposure Holders . . . Safelight Lamps and Filters . . . Identification Printer . . . Processing Hangers . . . Liquid and Powder X-ray Developers, Fixers, and Replenishers . . . Electric Chemical Mixers . . . Thermometers . . . Film Corner Cutter . . . Illuminators.

"KODAK" IS A TRADE-MARK

EASTMAN KODAK COMPANY, *Medical Division*, Rochester 4, N. Y.

Kodak



JUST MAIL IT TO "CANCER"
Give as generously as you can—today. Your check or money order in an envelope addressed to "Cancer", care of your local post office, will be delivered to the American Cancer Society office in your state.

Here's my $.....to help conquer cancer
NAME.....
ADDRESS.....
CITY.....ZONE.....STATE.....

Give and keep giving to help science defeat the disease that strikes, on the average, one out of every two homes in America. Say to yourself . . . here is life-giving money to help those stricken by Cancer to live again.

EVERY NICKLE AND DIME *I give helps support an educational program teaching new thousands how to recognize Cancer and what to do about it.*

EVERY QUARTER *I give helps set up and equip new research laboratories where scientists are dedicating their lives to find the cause—and cure of Cancer.*

EVERY DOLLAR *I send helps buy new equipment, helps establish new facilities for treating and curing Cancer, both still pitifully scarce in this country . . .* Guard those you love! Give to conquer Cancer!

AMERICAN CANCER SOCIETY

In Digestive Upsets

Gastro-intestinal disturbances frequently interrupt progress in tuberculosis. In such periods, Ovaltine in milk, a multiple dietary supplement, is especially useful in the nourishment of the patient.

1. **The protein** of this delicious food drink—Ovaltine in milk—is of high biologic value, supplies all the indispensable amino acids required for tissue maintenance and growth and other physiologic needs.

2. **Its contained vitamins and minerals** provide excellent amounts of vitamins A and D, ascorbic acid, niacin, riboflavin, thiamine, calcium, copper, iron, and phosphorus.

3. **Its carbohydrate energy** is promptly available for utilization.

4. **Its easy digestibility** makes for ready absorption of its valuable nutrients.

5. **Its delicious flavor,** appealing alike to children, adults, and the aged, makes it acceptable even when other foods may be refused.

6. **Its multiple nutrients,** in kind and amount, make Ovaltine in milk a highly efficient dietary supplement.

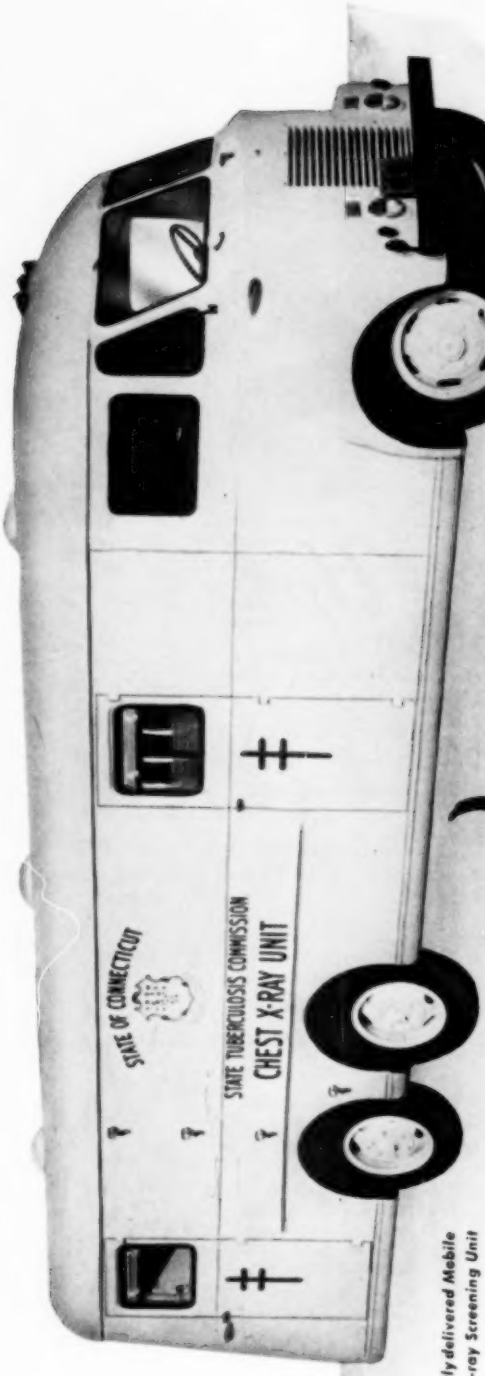
THE WANDER COMPANY, 360 N. MICHIGAN AVE., CHICAGO 1, ILL.

Ovaltine

Three servings daily of Ovaltine, each made of ½ oz. of Ovaltine and 8 oz. of whole milk, provide:*

CALORIES	676	VITAMIN A	3000 I.U.
PROTEIN	32 Gm.	VITAMIN B_1	1.16 mg.
FAT	32 Gm.	RIBOFLAVIN	2.0 mg.
CARBOHYDRATE	65 Gm.	NIACIN	6.8 mg.
CALCIUM	1.12 Gm.	VITAMIN C	30.0 mg.
PHOSPHORUS	0.94 Gm.	VITAMIN D	417 I.U.
IRON	12 mg.	COPPER	0.5 mg.

**Based on average reported values for milk.*



A recently delivered Mobile Chest X-ray Screening Unit

A Picker representative can save weeks of data-digging for any TB specialist or group interested in TB case-finding survey work. He'll have scaled plans for truck or trailer installations, reflecting the seasoned experience and latest thinking of authorities in Public Health work. And full specifications for Minograph equipment programs at various levels and goals: with cost figures complete down to the last penny.

PICKER Minograph

x-ray apparatus for chest screening

something to remember...

T. B. x-ray surveying is more than figures on charts: it's dealing with people. Technicians who have worked with all sorts of apparatus tell us that the Picker Minograph allows them to handle more people, more efficiently, more *humanly*. Let your local Picker representative explain its many operating advantages which promote subject co-operation, building good-will for your program, rather than bad tempers.

PICKER X-RAY CORPORATION
300 Fourth Ave., New York 10, N. Y.

ALUM ROCK SANATORIUM

SAN JOSE, CALIFORNIA

Telephone: MAYFAIR 4921

A non-profit sanatorium for the treatment of Tuberculosis and other diseases of the chest. It is ideally situated in the eastern foothills overlooking the Santa Clara Valley.

Medical Director
Buford H. Wardrip, M.D.
Associate Medical Director
C. G. Scarborough, M.D.

Visiting Medical Staff:

Harold Gayen Trimble, M.D., Oakland
Cabot Brown, M.D., San Francisco
J. Lloyd Eaton, M.D., Oakland
Ina Gourley, M.D., Oakland
Glenroy N. Pierce, M.D., San Francisco
Gerald L. Crenshaw, M.D., Oakland
James Robert Wood, M.D., Oakland

CHICAGO FRESH AIR HOSPITAL

A non-profit institution fully equipped for the care and management of diseases of the chest including treatments in the Emerson Lung Immobilizing Pressure Chamber.

Ideally located in a 20-acre park, accessable to bus, street car and elevated lines.

HUGO O. DEUSS, M.D., *Medical Director*
Telephone—Rogers Park 0321
2451 W. Howard Street at Western Ave. Chicago 45, Illinois

Maryknoll
SANATORIUM

(Maryknoll Sisters)

MONROVIA, CALIFORNIA

• A Sanatorium for the treatment of tuberculosis and other diseases of the lungs. Located in the foothills of the Sierra Madre Mountains, Southern exposure. Accommodations are private, modern, and comfortable. Rates are moderate. General care of patient is conducive to physical and mental well being.

SISTER MARY ELLEN
Superintendent

E. W. HAYES, M.D.
Medical Director

TUBERCULOSIS PHYSICIANS

from the United States are cordially invited to the **Commonwealth & Empire Health & Tuberculosis Conference,** to be held in **London, July 5 to 8, 1949.**

There will be seven scientific and clinical sessions—visits to hospitals and sanatoria—an Exhibition of X-ray apparatus.

$13.00 for four days

Details from:

Harley Williams, M.D.
Tavistock House North
London, W.C. 1. England

NATIONAL ASSOCIATION FOR THE PREVENTION OF TUBERCULOSIS

"The Beginning of

An Answer to the

Problem of Tuberculosis"

PARASAL

Panray's brand of PAS
(Para Amino Salicylic Acid)

is now available in quantities adequate for investigational use

"In all cases where P.A.S. has been tried the picture has followed the same pattern—loss of toxicity, control of pyrexia and cough, increased appetite, and gain in weight. Some of the patients are now fit for surgical treatment. The remainder, unsuitable for surgery, will soon present the problem of what is to be done with them. Will they get along as inactive open cases, or will further courses of P.A.S. be needed to ensure a reasonable span of life? I feel that in earlier cases, with less gross destruction of the lung, P.A.S. may well afford a complete cure. It also seems that the daily dose must be greatly increased—to not less than 20–25 g.—and must be continued for at least 3 months if better results are to be achieved. Local treatment with 1–2% spray should also be included. Probably a P.A.S. "umbrella" will reduce the risk of spread or reactivation through operation or during pregnancy. It thus seems that P.A.S. may be regarded as the beginning of an answer to the problem of tuberculosis . . ."

Erdei A.: The Lancet, July 17, 1948 P. 119

Write for bibliography, prices, and other pertinent data

Manufacturers:
HEXAGON LABORATORIES INC.

Sole Distributors:

THE PANRAY CORP.

Customs Manufacturers of Fine Organics

398 Broadway New York 13, N. Y.